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**Urban pollen rain: the local and extra-
local contribution to the airborne pollen
record in Florence (Italy)**

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CICLO XXXII

COORDINATORE Prof. Barbuji Guido

La pioggia pollinica urbana: il contributo locale ed extra-locale alla registrazione di polline aerodiffuso su Firenze (Italia)

Settore Scientifico Disciplinare BIO/02
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Index

1. Summary -----	3
2. Introduction -----	5
3. Comparing pollen data from moss cushions and a volumetric sampler: the study in the city of Florence (Italy) -----	8
4. The pollen rain in an urban environment: assessing the effect of nearby green areas -----	32
5. Cupressaceae pollen: new data about diffusion, record and preservation -----	58
6. Contribution of land cover and wind to the airborne pollen recorded in a South-European urban area -----	72
7. Allergenic airborne pollen in Florence (Italy): a record of 22 years -----	107
8. Airborne herbaceous pollen in Florence (Italy) -----	115
9. Conclusions -----	123
10. References -----	126
11. Ph.D. activities -----	139

1. Summary

The researches of this thesis focused on the pollen rain on the city of Florence (Italy) studied through the use of different methods of analysis: gravimetric (moss cushions) and volumetric traps (Hirst type samplers). The comparison between the two types of pollen sampling allowed to evaluate the accumulation time of pollen grains in the mosses, the quantitative/qualitative differences among the pollen data and the local and extra-local contribution to the pollen amounts, focusing in particular on cypress trees and the allergenic component of the pollen rain.

Moss cushions operate a continuous sampling over time, collecting pollen grains for a time interval greater than five years. The overall composition of the pollen rain is similar in both the sampling types, with noticeable differences from a quantitative point of view. The greatest local contribution is due to the arboreal component which displayed a strong proximity effect to the pollen values recorded at ground level. This is especially true for the most widespread ornamental trees, although the data interpolations show that pollen accumulation is uneven across the city. Regarding the extra-local contribution, the plants growing on the hills north of the city provide the greatest pollen amount in Florence, especially when the wind blows at a high intensity. The detailed investigation on Cupressaceae pollen grains allowed to estimate their accumulation rate at ground level and the proximity effects of these plants on the pollen record. Cypress tree pollen is the main component of the allergenic pollen rain, although herbaceous plants pollen concentrations are high over the years.

1. Riassunto

Le ricerche che compongono questa tesi hanno come argomento la pioggia pollinica nella città di Firenze studiata mediante differenti metodologie di analisi: trappole gravimetriche (cuscinetti di muschio) e volumetriche (campionatori di tipo Hirst). Attraverso queste due tipologie di campionamento è stato possibile stimare il tempo di accumulo dei granuli pollinici nei muschi, le differenze quantitative/qualitative ed il contributo locale ed extra-locale sulle quantità di polline, con particolare riferimento ai cipressi ed alla componente allergenica della pioggia pollinica.

I cuscinetti di muschio raccolgono la pioggia pollinica in maniera continuativa, accumulando granuli per un intervallo maggiore di cinque anni. La composizione della pioggia pollinica è relativamente simile nelle due metodologie di campionamento, mentre si osservano differenze notevoli da un punto di vista quantitativo. Il maggior contributo locale è dovuto alla componente arborea che mostra un elevato effetto di prossimità sui valori pollinici registrati al suolo. L'interpolazione dei dati ha mostrato che l'accumulo di polline è irregolare dentro la città e confermato una forte correlazione con la presenza degli alberi ornamentali più diffusi in ambito urbano. Per quanto riguarda l'apporto extra-locale, le piante che crescono sulle colline a nord della città forniscono il contributo di polline più elevato, soprattutto quando il vento spira ad intensità elevate. Lo studio sui granuli pollinici di Cupressaceae ha permesso di stimare il loro tasso di accumulo a livello del suolo e l'effetto prossimità di queste piante sulle registrazioni. Il polline di cipresso è la maggiore componente della pioggia pollinica allergenica, sebbene le concentrazioni di polline di piante erbacee siano e si mantengano elevate nel corso degli anni.

2. Introduction

Green spaces are a key element of the urban contexts, where they can limit the increasing environmental stresses (air pollution, excessive noise, etc.) and provide social well-being benefits (Tzoulas et al. 2007; Latinopoulos et al. 2016) improving environmental quality. The recent interest in the vertical gardens underlines the trend “to re-image the shape of the modern cities” (Abel 2010) introducing green spaces even in the middle of high buildings, in absence of available horizontal spaces.

On the other hand, the cultivation of a high number of plants, often selected among a few species, can lead to collateral problems for citizens. Plants release spores, pollen, seeds/fruits and other biological structures that may float in the air reaching different distances from the source. Airborne pollen grains are one of the main topics of the aerobiological studies, which are carried out to monitor the passive transport through the air of biological particles (Oteros et al. 2013), because they are able to cause allergic syndromes: the inhalation of pollen grains induces respiratory allergies in sensitive individuals, which are manifested as rhinitis and bronchial asthma (Gioulekas et al. 2004). The extensive use of ornamental species, often concentrated in restricted areas and flowering at the same time, may cause allergic sensitization in the city inhabitants (Cariñanos et al. 2017). Recent studies have shown that subjects resident in urban areas display a greater risk to contract respiratory disease compared to people residing in sub-urban areas (Antonicelli L. et al 2013). Moreover, the warmer climate and the high level of CO₂ in the cities affect plants physiology, inducing in early blooms and extended flowering periods (Ziello et al. 2012; Massetti et al. 2015).

In this context, aerobiological studies lead in urban environments becomes of great interest to evaluate the real contribution of the ornamental trees to the total airborne pollen, providing useful information for future urban planning and management.

The amount of spores and pollen grains that falls in a place for a given period of time is called “pollen rain” and directly reflects the vegetation of the surroundings. After transport in the atmosphere, whose

distance mainly depends on the meteorological variables, pollen grains fall and accumulate on various type of substrates, where may be preserved for a long time.

Moss cushions are among the most appropriate substrate to carry on an actual pollen analysis: they act as a gravimetric “natural trap”, preserving the pollen grains in a humid and acid medium. Moss cushions are widespread in many environments, including the urban ones, thanks to the ability to colonize a variety of habitats: this feature allows to gather simultaneously numerous samples in the study area. Thanks to the constant accumulation of pollen, they provide an average image of the pollen rain of many years, mitigating the differences of annual pollen production. Therefore, moss samples are widely used also as “modern analogues”, an informative tool to interpret fossil pollen spectra and to relate the pollen rain to the surrounding vegetation (Räsänen 2001; Graf & Chmura 2006).

The rate of pollen accumulation/preservation of mosses has not yet been definitively clarified: previous studies indicated that they record up to 15 years of pollen rain. Despite they furnish a reliable image of the pollen rain, they not always represent the appropriate methodology for all of the actual palynological studies.

Another type of pollen trap commonly adopted for quantitative/qualitative studies is the volumetric one, which provides accurate temporal registration of the airborne pollen, *i.e.* referable to definite time intervals. This method is based on the capture by impact of atmospheric particles on a surface through the aspiration of air. The sampler aspirates a known volume of air (10 l/min, approximately the lung capacity of an adult man) that passes over a plastic tape mounted on a rotating drum. The drum rotates at a regular speed, with an autonomy of seven days; an adhesive solution is applied to the plastic tape. At the end of sampling, the tape is cut, colored and observed under light microscope. The volumetric traps allow to draw up pollen calendars temporally detailed, particularly useful for prevent pollinosis symptoms.

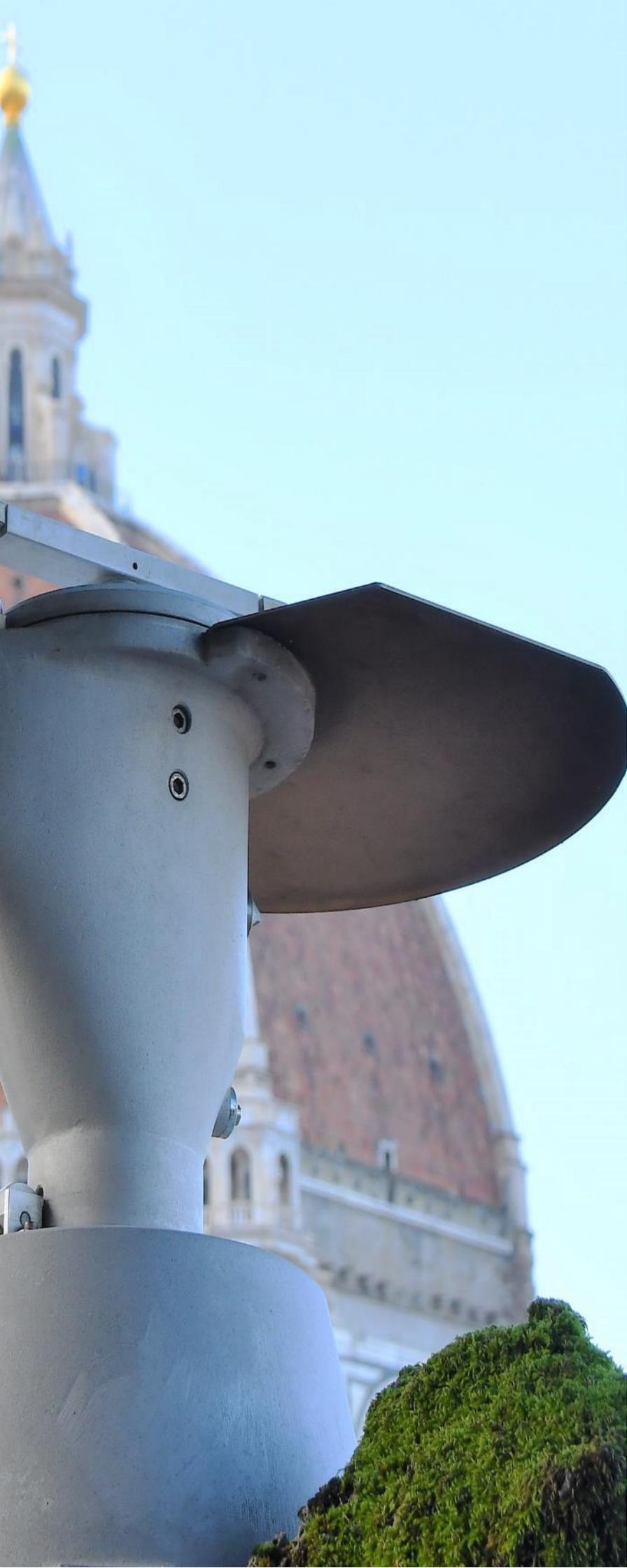
In this thesis, two different sampling methods (gravimetric and volumetric) were used to monitor the airborne pollen in the city of Florence. Starting from pollen data recorded by moss cushions, the work deal with defining the real representativeness of these data from a qualitative and temporal point of view by comparing the data collected by the use of the two different pollen traps (Chapter 3).

The pollen records by the network of gravimetric traps (mosses) have been related to the extension and composition of the urban green areas inside the historical center of Florence (Chapter 4) and this kind of analysis has been then applied for estimating the pollen diffusion from the most widespread family plant of urban and extra-urban area (Chapter 5).

The pollen data recorded in the city has been related to the land cover of the surroundings and meteorological variables to assess the areas of greatest pollen contribution in the city (Chapter 6).

In Chapter 7 the study focused on the pollen families with the highest allergenic potential. Finally, thanks to the use of a new volumetric trap, the airborne herbaceous pollen in the center of Florence has been studied (Chapter 8).

The topic of this work was the study of the airborne pollen detected in an urban environment through the use of two different sampling methods. Pollen data were related to the plant distribution in the surrounding environment to assess the local and extra-local contribution to the pollen rain detected in the city center of Florence.



3. Comparing pollen data from moss cushions and a volumetric sampler: the study in the city of Florence (Italy)

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Abstract

In Italy, aerobiological monitoring is usually carried out by the Regional agencies for environmental protection (ARPA) using volumetric samplers. Another widespread method for the study of airborne pollen is the analysis of moss cushions, generally used to relate pollen rain to flora and vegetation. In this study, we compare the data coming from these two pollen monitoring methods: volumetric samplers and moss cushions. The study was carried out in the city of Florence (Italy) and took into consideration two different spans of time. The two pollen monitoring methods show quantitative and qualitative differences. The main discrepancy seems to be the direct consequence of the different preservation time of the grains in the moss cushions depending on the features of the pollen grains. The study also provides an estimate of the period of pollen accumulation and preservation in moss cushions, which turned out to be more than 5 years. The two sampling methods furnish complementary information, volumetric samplers especially suited for aerobiological studies and moss cushions for environmental and palaeobotanical research.

Keywords: airborne pollen; pollen monitoring; urban pollen deposition; moss sample

1. Introduction

Aerobiological studies are carried out to monitor the passive transport of biological particles through the air (Oteros et al. 2013). They deal, in particular, with the detection of pollen grains causing allergic symptoms and the drawing up of pollen calendars. The inhalation of pollen grains induces respiratory allergies in sensitive individuals, which are manifested as rhinitis and bronchial asthma (Gioulekas et al. 2004). These studies are conducted by means of volumetric samplers generally located in urban areas, where the anthropogenic sources of gasses and particle emissions in the atmosphere are concentrated and people suffer more pollen allergies than in rural areas (Cariñanos & Casares-Porcel 2011).

In Italy, aerobiological monitoring is carried out by the Regional agencies for environmental protection (ARPA), which are Italian public institutions, and other pollen monitoring stations of the AIA network (Associazione Italiana di Aerobiologia). In Florence, Italy, ARPAT (Regional Agency for environmental protection of Tuscany) constantly monitors pollen and fungal spores by means of a volumetric sampler of the Hirst type (Hirst 1952) located in the north-eastern area of the town, very close to the city center.

Another way to monitor the airborne pollen and spores is the analysis of moss cushions. These substrates act as a natural pollen trap because of their slow growth, high water content and low pH that allow an excellent preservation of the grains (Wilmshurst & McGlone 2005). Mosses are widespread in many environments, including urban ones, thanks to their ability to colonize a variety of substrates. Over the years, numerous qualitative and quantitative surveys of the pollen rain have been carried out through the examination of mosses in many regions of Italy and also in urban areas such as in Bologna (Bertolani Marchetti et al. 1980), Turin (Caramiello Lomagno et al. 1983), Modena (Ferrari et al. 1986), Genoa (Montanari et al. 1986; Voltolini et al. 2000) and Perugia (Romano et al. 1988; Fornaciari et al. 1996). Previous data on the pollen rain in Florence concern the correlation between airborne pollen and vegetation (Zerboni et al. 1986), pollen diffusion above the

roof level and vertical variation (Mariotti Lippi & Mori Secci 1994; Mori Secci & Mariotti Lippi 1995).

Analyses of the pollen content of moss cushions are a common method for studying the relationship between the existing flora and vegetation and the current pollen rain. The knowledge of this relationship is a useful background for a correct interpretation of the past pollen spectra. In this perspective, modern-analogue studies (e.g. Gaillard et al. 1992; Court-Picon et al. 2005; Lazarova et al. 2006) are frequently carried out: they provide a useful tool for a detailed reconstruction of the vegetation history and about the interaction between humans and plants (Bellini et al. 2015).

The true period of pollen accumulation and preservation in mosses is not well defined: Caseldine (1981) and Cundill (1991) suggest a period of at least two years, while Bradshaw (1981) of about five years. Mulder and Janssen (1998) extended it to a period of about 10 years of pollen accumulation, while Crowder and Cuddy (1973) suggest that this period is from five to fifteen years. It is to note that these studies were mainly performed in north-eastern Europe where the climatic conditions differ from those of the southern European, possibly influencing the cushions growth rate (Pardoe et al. 2010) and consequently their capacity to accumulate and preserve the pollen grains.

In this paper, the pollen record from the moss cushions sampled in the historical center of Florence was compared with the airborne pollen detected by the volumetric sampler of ARPAT in Florence, for evaluating the difference in the results that come from the pollen data recorded by the two sampling methods, also relating it with the vegetation of the surroundings. Moreover, the comparison provides information about the time period of pollen accumulation and preservation in the moss cushions.

2. Materials and methods

2.1 Study area

The city of Florence (43°47'14"64 N, 11°14'59"64 E, 50 m a.s.l.) (Figure 1A) is located in north-eastern Tuscany, central Italy. The city center lays in the middle of the Arno River basin bordered to

the north-east by the hills of Careggi, Quarto, Fiesole and Settignano and to the south from the slopes of Bellosguardo, Arcetri and Pian de' Giullari. Florence's climate is continental temperate, with dry and very hot summers and mild/cold winters. The annual average temperature is 15.5° C: the coldest month is January (average temperature 6.8° C), the hottest is July (average temperature 25.3° C). The average annual rainfall is around 830 mm. In the last thirty years, the wettest month has been November (average of 105.4 mm) (Borchi & Macii 2015).

The urban green areas are mainly represented by cultivated trees and shrubs growing along the roads and in the public and private parks. The most used tree for ornamental purposes is *Tilia* sp. (15% of the total road trees), followed by Cupressaceae trees (14%) and *Quercus* sp. (13%) (Municipality of Florence, Territorial Information System, 2016). Meadows occur in the parks and along the river banks and lake shores. The largest park in the city, *i.e.* Cascine Park, hosts extensive meadows characterized by numerous species of high conservation importance (Mosti 2005). Along the Arno River, willows (*Salix alba* L.) and poplars (*Populus nigra* L.) grow in small thickets and are often accompanied by ruderal and nitrophilous herbaceous plants (mainly *Urtica* spp. and *Artemisia* spp.) (Foggi et al 2008).

The territory surrounding Florence is characterized by a mixture of rural areas and natural or semi-natural vegetation. The rural area mainly consists of vineyards and olive groves, sometimes accompanied by crops. *Quercus*-dominated woodlands are associated with coppices of *Ostrya carpinifolia* Scop and *Fraxinus ornus* L. mainly in the southeastern reliefs (Arrigoni & Foggi 1988). Close to the town, few grasslands are in a state of abandonment. In the past years, reforestation with pine and cypress trees strongly affected the slopes surrounding Florence (RaFT 2005). Cupressaceae trees (mainly *Cupressus sempervirens* L.) are also very common near villas and in the residential areas.

2.2 Sampling and treatment of the moss cushions

A total of sixteen moss cushions were randomly collected in the city center of Florence in 2008 (Figure 1B). The sampling was repeated at the same sites in 2017. All the 32 moss cushions have been sampled at ground level paying attention to prevent contamination by trampling. Single moss cushions of *Tortula muralis* Hedw were selected for sampling. When *T. muralis* was not found, other similar cushions were collected: *Thuidium tamariscinum* (Hedw.) Schimp., *Rhytidiadelphus triquetrus* (Hedw.) Warnst., *Racomitrium canescens* (Hedw.) Brid. Mosses identification was based on literature (Cortini Pedrotti 2006; Aleffi 2008).

Before laboratory treatments, the upper part of the cushions was separated from the basal one which was mixed with soil. All the samples (ca 2 g dry weight) were initially boiled in a 10% solution of NaOH and sieved with a 25 µm meshes. The samples were then treated with 37% HCl and 70% HF solutions. After washing and dehydration in an ascending series of acetic acid concentration, the samples were treated following the acetolytic method (Erdtman 1960) and placed in water and glycerin 50% v/v solution. At least 30 µl for each sample were completely observed. The observation was carried out at l.m. operating at 400x and 630x. Pollen identification was based on literature (Punt et al. 1976 – 1996; Reille 1992–1998) and on comparisons with reference pollen collections.

In order to have a general overview of the pollen rain of the city, the pollen data recorded from the moss cushion samples (MS) were considered as a whole. MS percentages refer to the average values calculated among those recorded in the single moss samples collected in the same years (MS data).

AP (Arboreal Pollen) includes pollen grains of trees, shrubs and climbers; NAP (Non Arboreal Pollen) includes herbaceous taxa. Unidentified pollen grains were counted as NAP.

The pollen spectra were designed using TILIA software (Grimm 2004).

2.3 Comparison between moss cushion and volumetric sampler pollen records

The record from the volumetric sampler refers to the Florence ARPAT sampling station located on the roof (ca 20 m above ground level) of the main hospital of the city (Azienda Ospedaliero-Universitaria Careggi), in the northern part of the town. The data concerning the time intervals from 2000 to 2007 and from 2009 to 2016 are available on the ARPAT website

(<http://www.arp.at.toscana.it>). On the website, the records are documented as number of grains per cubic meter. To compare the pollen concentration from moss cushions, expressed in grains/gram, with the pollen data from the volumetric sampler, the values have been recomputed as percentages of the total.

Moreover, the acetolysis method (Erdtman 1960) allows a more detailed identification of the pollen grains, because almost all non-sporopollenin components are destroyed making the morphological characteristics of the exine easier to see. Due to the different levels of pollen identification in the two records (genera and families in the moss record; mostly families in the volumetric sampler record), the detailed lists of taxa from the moss cushions were reduced to the shorter list reported in the ARPAT website (Table I).

2.4 Land cover around the sampling sites

For each sample, the land cover (urbanized or green areas) and the plants growing inside a buffer of a 500-meter radius from the sampling sites were analyzed. For the detailed report of each sampling site see Supplementary Material, Table SI 1.

The sites are located in urbanized areas where the green patches are mainly represented by private or public gardens and parks and by the natural vegetation around water bodies. The widest green areas are Boboli Gardens, Torrigiani garden and Cascine Park. In these green areas, *Quercus* sp. and *Cupressus* sp. are the most numerous trees. Near the Arno River, *Populus* sp. and *Salix* sp. sporadically occur together with hygrophilous herbs (Foggi et al 2008). The main roads crossing the sampling area are mostly bordered by *Celtis* sp., *Tilia* sp. and *Quercus* sp.

The volumetric sampler is located near a wide green area, almost entirely olive groves. The nearby roads are mostly bordered by *Pinus* sp. and *Quercus* sp.

2.5 Statistical analysis

Statistical analyses were made between the average percentages from the moss cushions collected in the same year and the ARPAT data, taking into account the previous year and the average of two, three, four, five, six, seven and eight years before the moss collection (Table II). The analysis was carried out to search the best relationship between the airborne pollen recorded by the volumetric sampler (the explanatory variable) and the pollen content deposited in the moss samples (the response variable).

MS refer to the average pollen content of the moss cushions collected in the same year (2008 and 2017); VS refers to the airborne pollen detected by the volumetric sampler during one year or the mean values of two or more years.

Linear regression models were applied using R-Studio software (R Core Team 2018) to evaluate the better correlation between volumetric sampler pollen records and moss samples according to the time intervals taken into consideration. The correlation between the two types of pollen records has been estimated evaluating P-value ($Pr > t$) and the coefficient of determination ($Adj. R^2$). Pearson correlation coefficient was also calculated.

Principal component analysis (PCA) was applied to estimate the relationships between the pollen taxa and the sampling methods and to test how pollen taxa explain the variance between the two datasets. PCA was conducted using the vegan package (Oksanen et al. 2013). All the pollen data were log-transformed to make data conform to normality: to test this assumption, the Shapiro-Wilk test was applied.

3. Results

3.1 2008 records

In the 2008 MS, Arboreal Pollen reached an average value of 70% of the total pollen sum (Figure 2). Considering the sampling site separately, AP exceeds half of the total grains in all of them except sites 2 and 6 (SI 2). Cupressaceae (24%), *Quercus* (16%) and Pinaceae (6%) were the most frequent. Cupressaceae pollen grains reached the highest value in the sampling site 12, *Quercus* pollen grains

showed the highest percentage in the site 3, Pinaceae pollen in the site 14 (SI 2). Among Non Arboreal Pollen, Poaceae (10%), Urticaceae (10%) and Asteraceae (5%) were the most abundant.

In the time interval 2000 – 2007, the volumetric sampler recorded an average AP value of 81% (Figure 2). Most of the AP belonged to Cupressaceae, with average percentages ranging between 45% and 57% (Figure 2). *Quercus* was the second most abundant taxon with average percentages of 7% - 12%. Pinaceae was the third most abundant taxon, with average percentages of 4% - 6% over the years; this pollen showed very different annual percentages (11% in 2001 and 2.5% in 2002). Pollen grains referable to NAP exceeded 20% only in few years, showing average percentages of 13% - 19% (Figure 2). Among these, Urticaceae was the most represented taxon, with average percentages ranging from 7% to 9%. Poaceae was the second most abundant taxon, with averages over years that varied between 3% and 5%. Asteraceae, Plantaginaceae and Amaranthaceae displayed low percentages (close to 1%).

3.2 2017 records

In moss cushions sampled in 2017, the majority of the pollen grains belonged to AP (average value 78%) (Figure 3). Considering the sampling site separately, only at the sampling site 5 AP and NAP percentages are quite similar (SI 3). Among AP, *Quercus* (25%) Cupressaceae (16%) and Pinaceae (14%) were the most abundant (SI 3). *Quercus* pollen grains reached the highest percentage in the sampling site 7, Cupressaceae in the site 10, Pinaceae in the site 14 (SI 3). Among NAP, Poaceae (7%) and Urticaceae (5%) were the most frequently represented families, followed by the Asteraceae family (3%).

In the years 2009 – 2016, VS records referred mainly to AP, with an average percentage over the eight years of 84% (Figure 3). Among these, Cupressaceae was the most represented family, with average percentages that ranged between 38% and 52% in the different periods. *Quercus* was the second most represented taxon, with average percentages ranging from 10% to 13%. Pinaceae family reached average values of 3 - 4 %. The average percentages of NAP ranged between 15% and 19%.

Urticaceae was the most represented family, with average percentages between 7% and 9%. Poaceae was the second most represented family, with averages between 3% and 5%. Other herbaceous taxa, like Asteraceae and Plantaginaceae, showed average values close to 0.5%.

3.3 Statistical analysis

Linear regression models showed an overall high statistical correlation (p value < 0.001) between MS and VS records of both data sets (Table III). In the 2008 records, the previous VS single year (2007) before the moss sampling showed the lowest correlation ($Adj. R^2$ 0.599). The higher correlations were the averages of seven years (2001-2007) ($Adj. R^2$ 0.727) and eight years (2000-2007) ($Adj. R^2$ 0.741). Indeed, all the averages of two - eight years displayed a strong correlation. The statistical results of 2017 records showed the lower correlation with the single year (2016) before the moss sampling ($Adj. R^2$ 0.531). The averages of five years (2012-2016) ($Adj. R^2$ 0.638) and eight years (2009-2016) ($Adj. R^2$ 0.636) showed the strongest correlation. Pearson correlation test confirmed these results for both data sets (Table III).

Regarding the graphical representation of the PCA, the first two axes are responsible for 99.2% of the total variance, respectively 95.4% and 3.8% in both datasets. In Figure 4, vectors show that the large part of the variance is explained by the most representative pollen taxa belonging to anemophilous plants, both arboreal (*Quercus*, Pinaceae) and herbaceous (Poaceae, Urticaceae): specifically, *Quercus* is the most representative pollen taxon in 2008 MS and Poaceae in 2017 MS. The other families and genera (with * in Figure 4) provided a very low level of information. The averages of the previous years before moss sampling were quite overlapped and more correlated with MS pollen records than the single years of VS (2007 and 2016 respectively).

4. Discussion

4.1 Comparison between the two pollen monitoring methods

The two records, from moss cushions and volumetric sampler, provide a fairly clear image of the airborne pollen in the city of Florence, reflecting the plants that are widespread in the urban area and surroundings. However, the records also show differences from a quantitative point of view. Arboreal pollen is always the most abundant, both in MS (around 75%) and in VS (more than 80%) of the two time intervals. Comparing the MS record with the VS record of the previous year only, the average value of MS in 2008 (70%) may be related to that of the VS in 2007 (87%). Higher values were recorded in MS 2017 (78,5) and in the years 2009-2016 by VS (average value 84%). Previous studies highlighted a greater accumulation of arboreal pollen in the moss cushion than in other kinds of pollen traps (such as the Tauber trap) (Spieksma et al. 1994; Räsänen et al. 2004; Lazarova et al. 2006; Lisitsyna et al. 2012).

A detailed analysis of the percentages of the single AP taxa in the two records (MS and VS) (SI 2, 3) evidenced that the discrepancy is mostly due to the amount of Cupressaceae pollen. This pollen taxon reaches higher pollen percentages in the MS (average close to 24% in 2008 and 16% respectively in 2017), than in the VS (45% in 2000–2007 and 52% in 2009–2016). The lower percentages of Cupressaceae recorded in mosses may be related to the breakage of their fragile wall (Spieksma et al. 1994) possibly during hydration (Duhoux 1982, Danti et al. 2011). This process is common in wet substrates and may reduce the preservation time of the grains in the moss cushions, causing their under-representation in comparison to the strip of the volumetric sampler. Despite this feature, the high pollen production of Cupressaceae (Hidalgo et al. 1999) is however appreciable by the values reached in the moss samples collected in proximity of these plants (SI 1, 2, 3), as already observed in a study carried out in Spain (Belmonte et al. 1999).

On the other hand, the pollen grains of *Quercus*, the second most represented taxon in both datasets, show a greater accumulation in MS (about 16% in 2008 and 25% in 2017) than in VS (about 10% in both time intervals), confirming a general better capacity of AP accumulation in mosses. In contrast to Cupressaceae, *Quercus* grains have a good resistance to degradation (Lisitsyna et al. 2012). This feature, combined with the widespread presence of these trees in the surroundings of MS sampling

points (SI 1) and the considerable specific gravity of its pollen grain (Bryant et al. 1989), could explain the higher pollen concentration recorded by the moss cushions, at ground level, than in the volumetric sampler, at 20 m above ground level.

The third most represented pollen grains in both records belong to plants of the Pinaceae family. This taxon reached about the same percentages in 2008 records but displays a greater accumulation in MS than VS in the 2017 records. A greater Pinaceae pollen concentration in moss cushions than in other pollen traps was already observed in previous studies (Vermoere et al. 2000; Räsänen et al. 2004; Pardoe et al. 2010). Similar pollen percentages of Pinaceae pollen showed by MS and VS in 2008 records may be due to the capacity of moss cushions to attenuate different annual rate of pollen production (Tonkov et al. 2001). In fact, during the time interval 2000-2007, VS recorded strong different annual values of Pinaceae pollen (*i.e.* 11% in 2001 and 2.5% in 2002, <http://www.arpat.toscana.it>). This result highlights how the study of moss samples provides a prolonged and accurate image over time but does not allow evaluating temporal or local variations (Vermoere et al. 2000). In spite of the widespread presence of these plants both in the urban and extra-urban area of Florence, the average percentages of Pinaceae pollen grains recorded by the volumetric sampler are lower than in other Mediterranean regions (Spain: Jato et al. 2000; Greece: Gioulekas et al. 2004). However, is not easy to quantify the local pollen contribution of Pinaceae due to the long range distance transport of their pollen grains (Szczepanek et al. 2017).

Among NAP, Poaceae, Urticaceae and Asteraceae show the highest percentages in MS and VS of both records. Poaceae pollen grains reach higher percentages in MS (10% in 2008 and 7% in 2017) than in VS (average values: 5% in VS 2000–2007 and 3.5% in VS 2009–2016). Urticaceae percentages are similar in MS and VS of 2008 records, while they are higher in the VS than MS in 2017 records. Asteraceae percentages are higher in MS of both datasets than VS. These results are in agreement with the scarce diffusion of pollen from the entomogamous plants.

Despite the differences, both types of pollen monitoring show a general decrease of herbaceous pollen grains over the years. This trend was already observed in other European areas and attributed to the substantial decrease in grasslands (D'Amato et al. 2007).

Finally, MS as well as VS show the prevalence of the AP grains in the pollen rain of Florence, as already observed in other Italian towns like Perugia (AP 71%, Romano et al. 1988) and Genoa (AP 65%, Guido et al. 1992), a result that underlines the relevance of the trees growing in the urban greenspaces (*i.e.* urban parks, street verges, cemeteries, gardens and sports grounds) and in the surroundings. These records differ from what Spieksma et al. (1994) observed in a similar study conducted in Leiden (Netherlands) where AP percentage was about 60% in moss samples and about 35% in volumetric samples. However, the geographical and topographical contexts of these towns are very different. The Leiden values seem to underline the importance of the pollen supply of the trees growing in the urban greenspaces, given that the surrounding territory is mainly exploited for herbaceous crop cultivation, while the territory and hilly slopes facing Florence, Genoa and Perugia are more or less extensively covered by tree cultivations and forested areas. As a consequence, the AP amount is here largely due to extra urban supply.

4.2 Pollen time deposition in the moss cushions

The statistical analysis showed that the overall correlation between the average percentage values from MS and the VS data is very high. The lowest correlation is shown when the MS values are compared with the VS data of the single previous year (MS 2008 with VS 2007; MS 2017 with VS 2016) making evident that the pollen accumulated in the moss cushions is not the airborne pollen of one year only. Increasing the number of years of VS record, the correlation becomes stronger, proving the capacity of moss cushions to preserve pollen over time and their overall representation of the pollen diffusion in a long span of time. The stronger correlation was observed comparing the moss pollen content 2008 with eight years of ARPAT records (2000–2007) and the moss pollen content

2017 with five years of ARPAT records (2012–2016) suggesting that the pollen content of the moss cushions reflects a period of pollen deposition lasting five – eight years.

PCA (Figure 4) confirms that the single year before the sampling does not offer the best representation of the total pollen content of the mosses: this is evident by the variance explained by the second principal component analysis axis. Interestingly, almost the total variance between the two sampling methods is given by some of the most represented taxa, such as *Quercus* in the 2008 records, Poaceae in the 2017 records. The correlation is also strongly provided by Pinaceae pollen in the 2008 records due to the similar value recorded during this time interval. On the contrary, the correlation is not supported by Cupressaceae and, to a lesser extent, Asteraceae: the former for the scarce representation in mosses, the latter for their scarce air diffusion.

Previous studies (Caseldine 1981; Cundill 1991; Spieksma et al. 1994; Räsänen et al. 2004; Lisitsyna et al. 2012; Lisitsyna & Hicks 2014) indicated a time of pollen accumulation and preservation of about 5-15 years. Our results are placed in the middle of this time interval, despite the different environmental contexts where these studies have been carried out. In fact, the data from literature are referred to boreal areas where the climatic conditions are different from those of the southern Europe and the pollen analyses were performed on mosses with a definite annual increment (Rasanen et al. 2004) and an optimal growth-form for pollen deposition (Pardoe et al. 2010).

5. Conclusions

The results of this study confirm that the overall composition of the pollen rain in an urban area is relatively similar in both moss cushions and volumetric sampling data. Quantitative differences between the two records are more conspicuous than the qualitative ones and are strongly affected by the different rate of preservation of the single pollen grains in the moss. Indeed, the proximity of Cupressaceae plants is well recorded by the moss samples, even if they are under-represented with respect to the MS records. The proximity of the source plants is even better shown by the

concentration of *Quercus* pollen, which is well preserved in the moss cushions. Regarding Pinaceae, the long distance transport of these pollen grains fades the “proximity effect” of the source.

The two types of sampling operate at different spatio-temporal scales. The results confirm that the volumetric sampler is more useful for allergenic surveys to safeguard public health, while the use of moss cushion is particularly useful for the study of the relationship between flora and pollen rain and well reflects the surrounding environmental context. The pollen content from moss samples covers a time span greater than five years, ensuring the continuous pollen deposition and, as a consequence, attenuating the different annual rates of pollen production. For this reason, moss samples provide essential information suitable for palaeobotanical researches and palaeoenvironmental reconstructions.

The two types of samplers can be used in parallel providing data that integrate with each other; the present study confirms that the VS records evidence temporal changes and fluctuations on the background of the MS record.

Figure 1: Geographical location of Florence (A); position of the sampling sites (B): volumetric sampler (VS) and moss cushions (numbers).

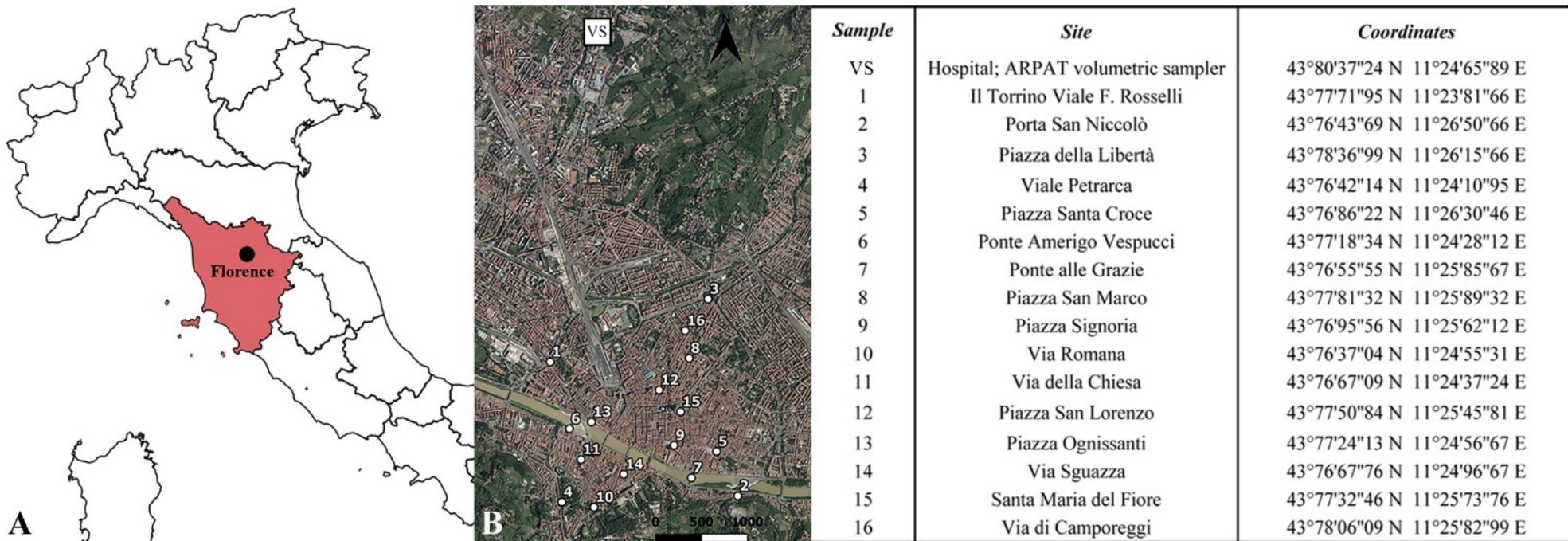


Figure 2: Pollen percentages of 2008 records comparing the average percentages from the moss cushions collected in 2008 and the data from the volumetric sampler from 2000 to 2007 (previous year and the average of two, three, four, five, six, seven and eight years before the moss collection).

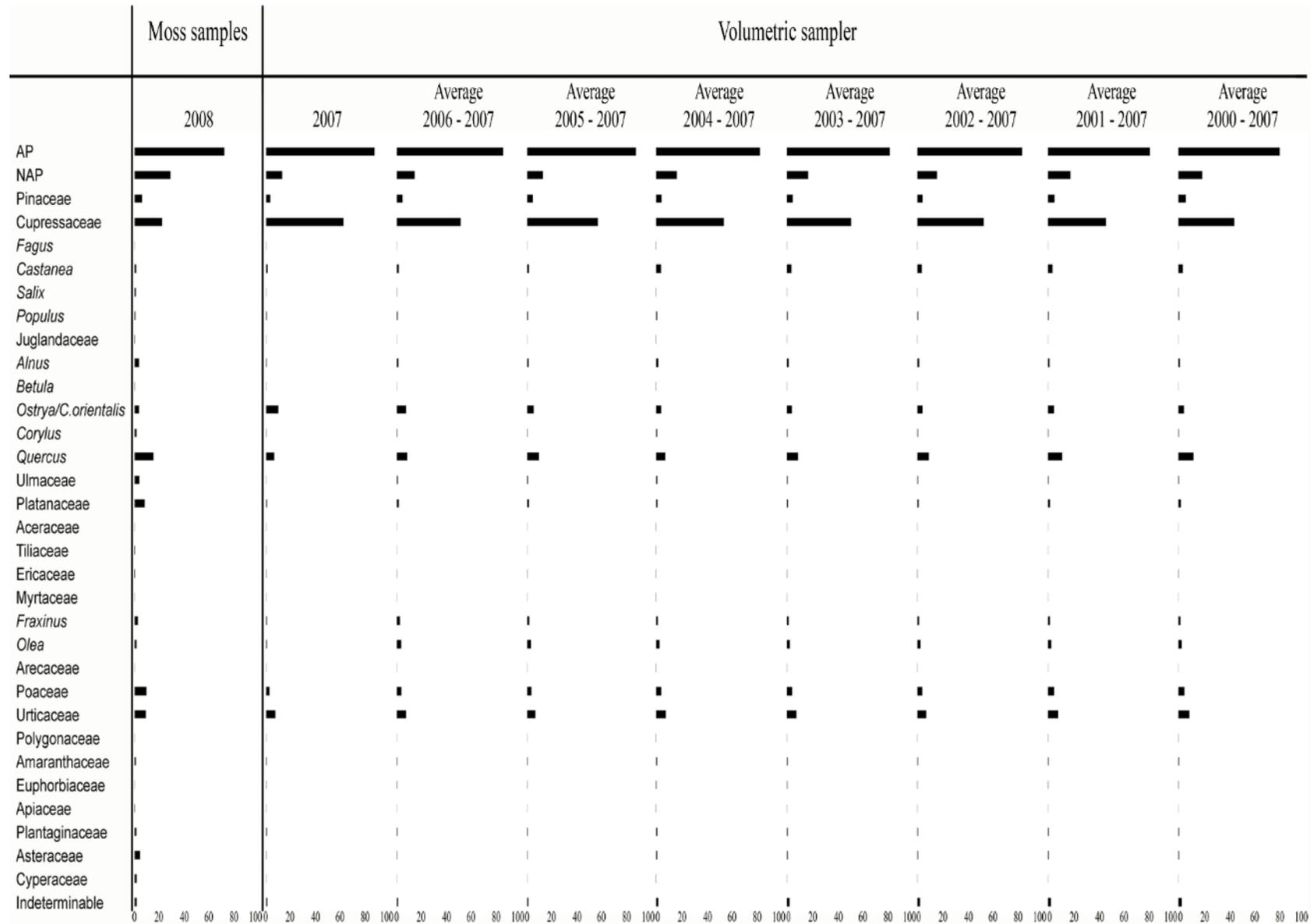


Figure 3: Pollen percentages of 2017 records comparing the average percentages from the moss cushions collected in 2017 and the data from the volumetric sampler from 2009 to 2016 (previous year and the average of two, three, four, five, six, seven and eight years before the moss collection).

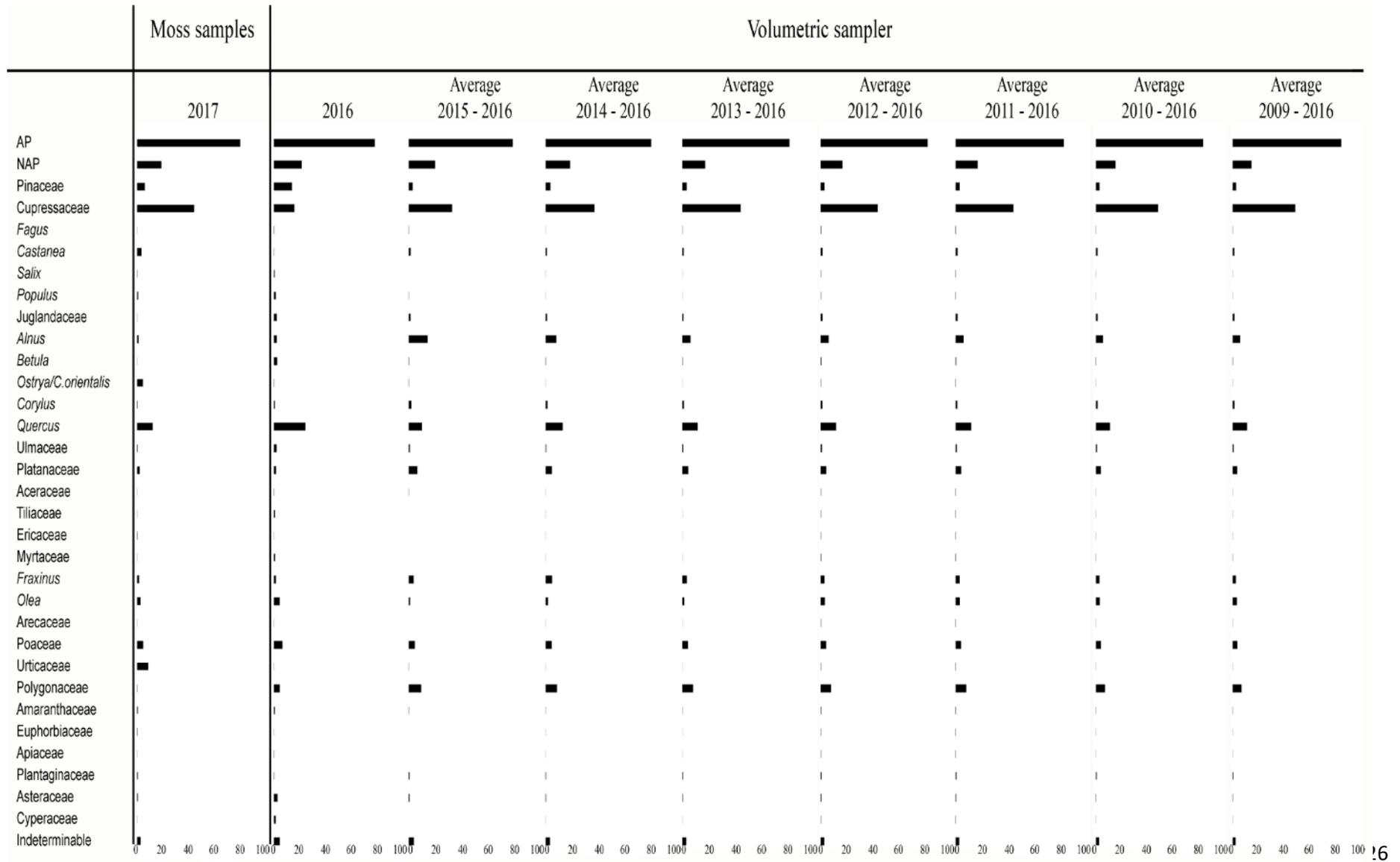


Figure 4: Graphical representation of PCA results. With the symbol * are marked the families and genera that provided a very low level of information.

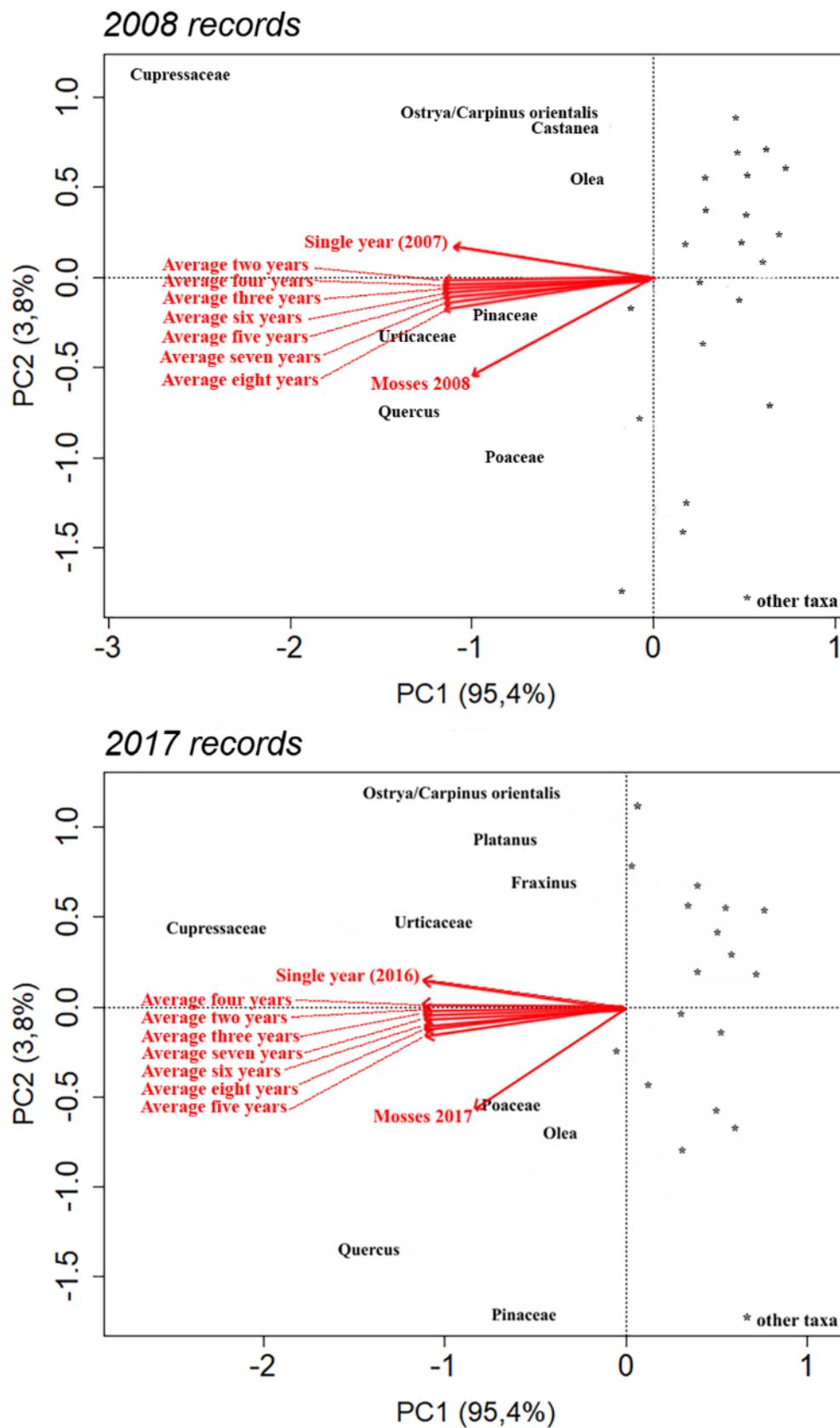


Table I: Pollen families reported in the ARPAT spectrum and corresponding pollen taxa identified in the moss samples.

<i>VOLUMETRIC SAMPLER (VS)</i>	<i>MOSS SAMPLES (MS)</i>
Pinaceae	<i>Abies</i> sp. <i>Cedrus</i> sp. <i>Pinus</i> sp.
Juglandaceae	<i>Juglans</i> sp. <i>Carya</i> sp.
<i>Quercus</i> sp.	<i>Quercus ilex</i> L. deciduous <i>Quercus</i>
Ulmaceae	<i>Celtis</i> sp. <i>Ulmus</i> sp.
Ericaceae	<i>Erica</i> sp. <i>Arbutus</i> sp.
Myrtaceae	<i>Myrtus</i> sp. <i>Eucalyptus</i> sp.
Urticaceae	<i>Parietaria</i> sp. <i>Urtica</i> sp.
Plantaginaceae	<i>Callitriche</i> sp. <i>Plantago</i> sp.

Table II: Time interval of moss samples and volumetric sampler data statistically compared in the two datasets. Moss samples (MS) values refer to the average of the sixteen moss cushions collected in each year of sampling (2008 and 2017). Volumetric sampler (VS) data refer to the single year before mosses sampling and the average of following previous years.

<i>MOSS SAMPLES (MS)</i>		<i>VOLUMETRIC SAMPLER (VS)</i>
		2007
		Average two years (2006-2007)
		Average three years (2005-2007)
<i>2008 records</i>	2008	Average four years (2004-2007)
		Average five years (2003-2007)
		Average six years (2002-2007)
		Average seven years (2001-2007)
		Average eight years (2000-2007)
		2016
		Average two years (2015-2016)
		Average three years (2014-2016)
<i>2017 records</i>	2017	Average four years (2013-2016)
		Average five years (2012-2016)
		Average six years (2011-2016)
		Average seven years (2010-2016)
		Average eight years (2009-2016)

Table III: Analysis of correlation between moss samples (MS) and volumetric sampler (VS) data in 2008 records and 2017 records. Significant level:

$p \leq 0.001$ (***). Av. = average. (at full page width)

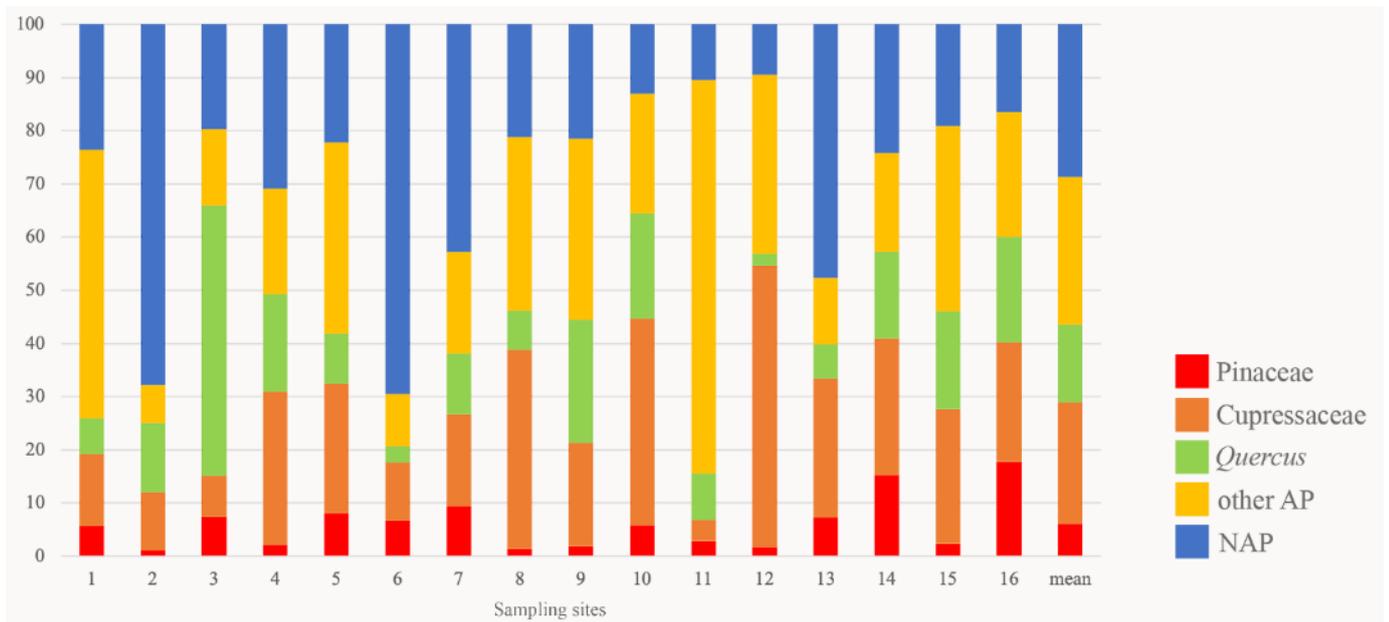
	<i>MOSS SAMPLES (MS)</i>	<i>VOLUMETRIC SAMPLER (VS)</i>	<i>Regression coeff.</i>	<i>S. Error</i>	<i>t value</i>	<i>Pr(>t)</i>	<i>Adj. R²</i>	<i>Pearson's coeff.</i>
<i>2008 records</i>	2008	2007	0.749	0.111	6.661	3.15e-07 ***	0.599	0.785
		Av. two years (2006-2007)	0.814	0.097	8.317	4.76e-09 ***	0.701	0.841
		Av. three years (2005-2007)	0.819	0.097	8.371	4.17e-09 ***	0.704	0.834
		Av. four years (2004-2007)	0.831	0.100	8.318	4.75e-09 ***	0.701	0.842
		Av. five years (2003-2007)	0.828	0.097	8.505	3.02e-09 ***	0.711	0.845
		Av. six years (2002-2007)	0.827	0.098	8.437	3.56e-09 ***	0.707	0.841
		Av. seven years (2001-2007)	0.824	0.093	8.846	1.34e-09 ***	0.727	0.851
		Av. eight years (2000-2007)	0.831	0.090	9.155	6.51e-10 ***	0.741	0.855
<i>2017 records</i>	2017	2016	0.648	0.111	5.814	3.02e-06 ***	0.531	0.843
		Av. two years (20015-2016)	0.707	0.100	7.077	1.06e-07 ***	0.628	0.881
		Av. three years (2014-2016)	0.723	0.102	7.086	1.04e-07 ***	0.629	0.882
		Av. four years (2013-2016)	0.723	0.102	7.055	1.13e-07 ***	0.627	0.881
		Av. five years (2012-2016)	0.735	0.101	7.226	7.26e-08 ***	0.638	0.885
		Av. six years (2011-2016)	0.740	0.103	7.178	8.22e-08 ***	0.635	0.882
		Av. seven years (2010-2016)	0.739	0.103	7.169	8.41e-08 ***	0.634	0.881
		Av. eight years (2009-2016)	0.750	0.104	7.192	7.91e-08 ***	0.636	0.883

Table SI 1. Percentages values of the land cover surrounding the pollen sampling sites. Built-up areas mean streets, houses and any type of construction.

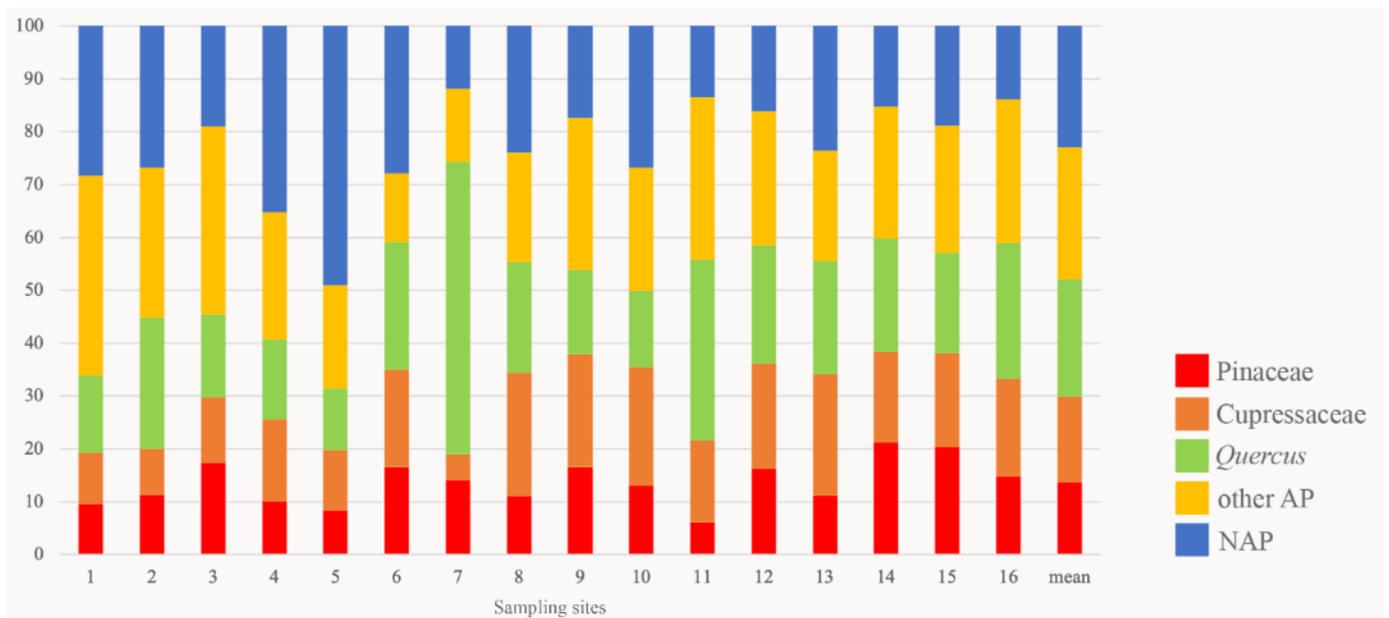
Green areas mean public/private gardens or parks. Among the main trees, the symbol ⁺ marks the most prevalent trees in the surrounding.

<i>Sample</i>	<i>Site</i>	<i>Built-up areas</i>	<i>Green areas</i>	<i>Water bodies</i>	<i>Main trees</i>
VS	Hospital	72.2	27.6	0.3	<i>Olea</i> sp. ⁺ ; <i>Pinus</i> sp.; <i>Quercus</i> sp.; <i>Platanus</i> sp.; <i>Cupressus</i> sp.
1	Il Torrino Viale F. Rosselli	90.5	9.5		<i>Pinus</i> sp. ⁺ ; <i>Tilia</i> sp.; <i>Fraxinus</i> sp.; <i>Cedrus</i> sp.
2	Porta San Niccolò	44.7	32.8	22.5	<i>Olea</i> sp. ⁺ ; <i>Quercus</i> sp.; <i>Cercis</i> sp.; <i>Prunus</i> sp.; <i>Cupressus</i> sp.
3	Piazza della Libertà	96.1	3.8	0.2	<i>Tilia</i> sp. ⁺ ; <i>Quercus</i> sp. ⁺ ; <i>Platanus</i> sp.; <i>Celtis</i> sp.
4	Viale Petrarca	74.0	26.0		<i>Celtis</i> sp. ⁺ ; <i>Quercus</i> sp. ⁺ ; <i>Platanus</i> sp.; <i>Tilia</i> sp.
5	Piazza Santa Croce	94.7	5.3		<i>Celtis</i> sp. ⁺ ; <i>Tilia</i> sp.; <i>Quercus</i> sp.; <i>Prunus</i> sp.; <i>Pinus</i> sp.
6	Ponte Amerigo Vespucci	65.7	4.1	30.2	<i>Platanus</i> sp. ⁺ ; <i>Cupressus</i> sp.; <i>Celtis</i> sp.
7	Ponte alle Grazie	62.8	9.8	27.4	<i>Tilia</i> sp. ⁺ ; <i>Quercus</i> sp.; <i>Pinus</i> sp.
8	Piazza San Marco	93.3	6.7		<i>Ulmus</i> sp. ⁺ ; <i>Magnolia</i> sp.
9	Piazza Signoria	99.4		0.6	
10	Via Romana	63.3	35.7	1.0	<i>Quercus</i> sp. ⁺ ; <i>Cupressus</i> sp.
11	Via della Chiesa	95.0	4.9	0.1	<i>Platanus</i> sp. ⁺ ; <i>Tilia</i> sp.; <i>Quercus</i> sp.; <i>Celtis</i> sp.; <i>Cupressus</i> sp.
12	Piazza San Lorenzo	99.7	0.3		<i>Cupressus</i> sp. ⁺ ; <i>Pyrus</i> sp.
13	Piazza Ognissanti	70.0	0.3	29.7	<i>Quercus</i> sp. ⁺ ; <i>Celtis</i> sp.
14	Via Sguazza	94.8	0.4	4.8	<i>Ulmus</i> sp. ⁺ ; <i>Magnolia</i> sp.; <i>Tilia</i> sp.
15	Santa Maria del Fiore	99.6	0.4		<i>Olea</i> sp. ⁺ ; <i>Magnolia</i> sp.; <i>Cedrus</i> sp.
16	Via di Camporeggi	99.6	0.4		<i>Olea</i> sp. ⁺ ; <i>Magnolia</i> sp.
	<i>mean</i>	83.2	10	6.8	<i>Olea</i> sp.; <i>Cupressus</i> sp.; <i>Celtis</i> sp. ⁺ ; <i>Tilia</i> sp.; <i>Quercus</i> sp.

SI 2: Pollen percentages in the moss samples collected at different sites in 2008. AP (dominating taxa are highlighted) and NAP.



SI 3: Pollen percentages in the moss samples collected at different sites in 2017. AP (dominating taxa are highlighted) and NAP.





4. The pollen rain in an urban environment: assessing the effect of green areas

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Abstract

Urban green areas can improve people's quality of life, despite airborne pollen may provoke allergic syndromes. The record of the pollen rain in different sites within a city can be useful to understand the dynamics of airborne pollen diffusion and deposition and the influence of the different plants. Moss cushions are natural gravimetric pollen traps and represent a useful and economic tool for this kind of survey. Several moss samples were collected in the same sites of the historical city center of Florence at the distance of a decade. In order to test the effect of urban green areas on pollen records, the land cover was analyzed in buffers drawn around each sampling site. Pollen frequencies were correlated with the extension of the green spaces and with the number of trees inside the buffers. Pollen concentration maps were drawn to plot the pollen diffusion at the different sites of the city center.

The pollen records at ground level displayed an uneven concentration across the city and a strong prevalence of arboreal pollen. The greatest statistical correlation between pollen frequencies and arboreal land cover was shown in the larger buffers. The influence of Cupressaceae and *Quercus* was noteworthy at great distance. Some of the trees commonly used along the main roads, like *Celtis* and *Tilia*, showed a strictly local influence on the pollen rain.

The results of the study highlight the utility of a network of records inside the towns for evaluating the actual contributions of the plants on the pollen accumulation at the height where people walk. They may provide useful indication for the planning of urban green areas in order to reduce health risks for citizens.

Keywords: Aerobiology; Ornamental plants; Pollen; Urban Ecosystem; Urban park

Cover image: The city center of Florence from the hill of Fiesole.

1. Introduction

Urban green spaces are a key element of the cities landscape, improving people's quality of life (Tzoulas et al. 2007; Latinopoulos et al. 2016) and then promoting public health, *i.e.* the state of complete physical, mental and social well-being (WHO 1948). On the other side, plants release pollen grains: as a consequence, the extensive use of ornamental species, often concentrated in restricted areas (*i.e.* monospecific roadside trees) and flowering at the same time, may cause allergic sensitization in the city inhabitants (Cariñanos et al. 2017). This is particularly relevant because people living in urban areas suffer more pollen allergies than those who live in the countryside (D'Amato et al. 2007). This condition is probably due to the jointed interaction between pollutants and pollen grains that often leads to a release of antigens resulting in an altered allergenicity (Bartra et al. 2007; Di Bucchianico et al. 2019). Moreover, the urban environment, that is warmer than the nearby areas, may induce early blooms and an overall extended flowering period that result in a longer exposition to allergenic pollen than in the countryside (Massetti et al. 2015).

In the last decades, an increase of the annual pollen index (*i.e.* the sum of average daily pollen concentrations over the year) was recorded across Europe (Ziello et al. 2012). This trend is possibly related to the increase of the greenhouse gas CO₂ due to the human activities, which affect the physiological performances of the plants (Ziello et al. 2012), as suggested by experimental studies carried out on *Ambrosia artemisifolia* L. (Wayne et al. 2002; Rogers et al. 2006).

In this context, aerobiological studies lead in urban environments becomes of great interest for evaluating the real contribution of the ornamental trees to the total airborne pollen, a knowledge that provide useful information for future urban planning and management.

Aerobiological surveys in urban areas are often performed through the use of one volumetric trap - frequently a 7-day volumetric spore trap (Hirst 1952) - placed inside the city, at roof level (about 20 m above ground level). These analyses lead to draw up detailed pollen reports by recording airborne pollen at specific time intervals. The trap captures the airborne pollen coming from a large surrounding area overlooking the presence of tall architectonic barriers that prevent the air flows

(Maya-Manzano et al. 2017) and the effect of the combination of horizontal and vertical surfaces arising from the arrangement of the buildings (Nunez and Oke 1977). In general, urban settlements are considered to reduce by some 20% the amount of pollen coming from outside the city (Gonzaloga-Garijo et al. 2006).

To overcome the limitation due to the use of a single sampling point, Werchan et al. (2017) used a network of gravimetric traps placed in the city of Berlin, Germany, at a height where people breathe for evaluating the real pollen impacts on allergy sufferers inside the urban area. In Wrocław (Poland), two volumetric samplers were contemporarily used (Bilińska et al. 2019).

The present study was conducted in Florence, Italy, using a network of natural gravimetric pollen traps, *i.e.* moss cushions, which were collected close to the ground level. Moss cushions are widespread in quite every context and are characterized by a slow growth and a low pH which allows an excellent preservation of the pollen grains (Wilmshurst and McGlone 2005). The time of pollen accumulation/preservation of the moss cushions has not yet been definitively clarified, ranging from two up to fifteen years (Crowder and Cuddy 1973; Caseldine 1981; Bradshaw 1981; Cundill 1991; Mulder and Janssen 1998; Ciani et al. 2019). Anyway, they provide an average image of the pollen rain, mitigating the effect of the annual variations due to the weather or other conditions (pruning, plant diseases, etc.). For this features, moss samples are also commonly used to determine the relationship between the existing vegetation and the current pollen rain, furnishing a fundamental tool for interpreting past pollen spectra, according to the modern-analogue technique (*e.g.* Gaillard et al. 1992; Court-Picon et al. 2005; Lazarova et al. 2006; Bellini et al. 2015). Surveys of the recent pollen rain through the analysis of moss cushions were performed in other Italian towns, such as Turin (Caramiello Lomagno et al. 1983), Genoa (Montanari et al. 1986; Voltolini et al. 2000), Modena (Ferrari et al. 1986), Bologna (Bertolani Marchetti et al. 1980), and Perugia (Romano et al. 1988; Fornaciari et al. 1996).

The main aim of this study is to examine the spatial diffusion of pollen within the urban area of Florence and to relate the pollen frequencies to the distribution of the source-plants. The moss

sampling was repeated twice to assess the possible changes of the pollen rain in the city at a distance of a decade.

2. Materials and methods

2.1. Study site

Florence (central Italy) is located in the Arno-river basin, a vast alluvial plain surrounded by hills and mountains of medium height (Figure 1A). The study was performed in the city center. The climate of Florence is sub-Mediterranean, characterized by dry summers, wet autumns-springs, and mild winters (Petralli 2011). The annual average temperature is 15.5° C: the coldest month is January (average temperature 6.8° C), the hottest July (average temperature 25.3° C). The average annual rainfall is 830 mm: in the last thirty years, the wettest month was November (average 105.4 mm) (Borchi and Macii 2015).

The urban green areas are mainly public and private parks or gardens and flowerbeds, in addition to the rows of ornamental trees and shrubs along the roads. The most common ornamental trees are *Tilia* L., *Cupressus* L., and *Quercus* L. (Table I). Patches of natural vegetation can be found along the Arno riverbed and in neglected parts of the largest parks (*i.e.* *Cascine Park*, *Boboli*).

The landscape surrounding the urban area is characterized by cultivation, in particular olive groves (Ciani et al., 2019). *Quercus*-dominated woodlands and very few grasslands are widespread on the hilly slopes facing the city. Coniferous or mixed coniferous/broad-leaved forests occur in reforested areas.

2.2. Sampling methods and pollen analysis

Sixteen moss cushions were collected at ground level in squares, streets and avenues, randomly chosen in the city center of Florence, and along the Arno river (Figure 1B). The sampling was conducted in 2008 and repeated in 2017 at the same sites, for a total amount of 32 samples.

Analyses were performed on the living part of the moss cushions to limit the effect of a differential pollen preservation (Bradshaw 1981). All the moss samples were treated following Ciani et al. (2019). Observation was performed at light microscope operating at 400-630x. Pollen identification was based on literature (Punt et al. 1976 – 1996; Reille 1992–1998) and comparison with reference pollen collections. In the text, Pinaceae only includes saccate pollen grains.

Pollen values are expressed as percentages and as absolute pollen frequency (APF= number of pollen grains per gram of sample). AP (Arboreal Pollen) includes pollen grains of trees, shrubs, and climbers; NAP (Non-Arboreal Pollen) includes herbaceous taxa.

2.3. Spatial and statistical analysis

Two types of spatial analyses were carried out in buffers of 125 m, 250 m, and 500 m radius (125m, 250m, 500m buffers) drawn around the sampling sites:

- Land cover analysis.

The territory was referred to three types of land cover categories: 1) built-up areas (buildings, streets and squares); 2) urban green areas (parks, gardens, meadows, etc.); and 3) water bodies (river, pools, etc.). In the city center, the height of the buildings does not overcome 20 meters, except for few monumental buildings, towers, and bell-towers.

Urban green areas were divided into two subcategories on the basis of the dominant plants growth form: trees or herbaceous species. Statistical analysis was applied to relate the total APF of each moss sample to the extension of the urban green areas (m²) inside each buffer. The APF of AP was related to the area covered by trees and the APF of NAP to the area covered by herbs.

- Tree point analysis.

The trees of public and private green areas around the sampling sites were geolocalized. Statistical analysis was performed to relate the APF of each tree pollen to the number of the corresponding source-plants in each buffer. Due to the impossibility of identifying the dominant herbaceous species, Principal component analysis (PCA) was performed on NAP values (for NAP pollen spectra see Ciani

et al., 2019) to test which families are most influential on herbaceous pollen concentrations recorded in the samples. PCA was conducted using the vegan package (Oksanen et al. 2013) with R-Studio software (R Core Team, 2018). All the pollen data were log-transformed to make data conform to normality: to test this assumption, the Shapiro-Wilk test was applied.

Both spatial analyses were made using ESRI ArcMap 10.6 software with WMS layers provided by the Territorial and Environmental Information System of Tuscany (<http://sit.comune.fi.it>) and integrated by the use of ortophotos. For the land cover analysis, the layers refer to 2013, the central year between the two years of sampling, assuming that negligible changes occurred in the urban green areas. For tree point analysis, the data refer to the 2016 layers. Both data have been updated by direct observations in the field. Ornamental trees were identified following Pignatti et al. (2019).

Statistical analyses were performed using repeated-measures analysis of variance (ANOVA) over the two years of sampling (2008 and 2017) in each site. All the pollen data were log-transformed to normalize the data. The analysis was performed using R-Studio software (R Core Team 2018).

In order to spatialize the pollen frequencies and to display the pollen diffusion inside the studied area, pollen concentration maps were drawn using inverse-distance weighting spatial interpolation. The graphic elaboration was made using ESRI ArcMap 10.6 software.

3. Results

3.1. Pollen analysis

3.1.1. 2008 samples

In the 2008 samples (Figure 2A), most of the pollen grains belonged to AP (average values 67.4%, APF 38,000 grains/gram): the highest value was reached at the site 11 (90.5%, APF 181,000 grains/gram), the lowest at the site 6 (29%, APF 11,000 grains/gram). Among AP, the most represented plants were Cupressaceae/Taxaceae (average values 22%, APF 10,500 grains/gram) that showed the highest value (51%, APF 45,000 grains/gram) at the site 12 and the lowest (4%, APF 8,000) at the site 11. The second most represented plants belong to *Quercus*, both evergreen and

deciduous (average value 14%, APF 8,000 grains/gram): the highest value was 50% (APF 34,000 grains/gram) at the site 3 and the lowest value 2% (APF 2,000 grains/gram) at the site 12. Pinaceae were the third most represented *taxon* (average percentage 6%, APF 3,000 grains/gram): the highest percentage (18%, APF 4,000 grains/gram) was recorded at the site 16, the lowest (1%, APF 1,000 grains/gram) at the site 2. Average values close to 4-5% were reached by Betulaceae, *Ostrya* Scop. /*Carpinus orientalis* Mill., *Ulmus* L., and *Platanus* L.; average values of 1-2% were reached by *Populus* L., *Olea* L. and other Oleaceae (*Fraxinus* L., *Ligustrum* L., *Phyllirea* L.).

NAP displayed an average value of 33% (APF 16,000 grains/gram): the highest (71%, APF 27,000 grains/gram) was reached at the site 6, the lowest (9.5%, APF 19,000 grains/gram) at the site 11.

3.1.2. 2017 samples

In the 2017 samples (Figure 2B), AP reaches an average percentage close to 74% (APF 63,000 grains/gram) with the highest value (89%, APF 163,000 grains/gram) at the site 3 and the lowest value (47%, APF 22500 grains/gram) at the site 6. Among these, the most represented *taxon* was *Quercus* (average 21%, APF 21,000 grains/gram) who reached the highest value at the site 3 (54%, APF 99,000 grains/gram), the lowest at the site 6 (11%, APF 5,000 grains/gram). The second most represented plants were Cupressaceae/Taxaceae (average percentage 15%, APF 12,000 grains/gram), the highest value (21%, APF 20,000 grains/gram) at the site 4 and the lowest value (5%, APF 10,000 grains/gram) at the site 3. Pinaceae (average percentage 13%, APF 11,000 grains/gram), displayed the highest percentage (21%, 25,000 grains/gram) at the site 14 and the lowest percentage (5%, APF 6,000 grains/gram) at the site 10. Average value of 7% (APF 5,000 grains/gram) was reached by Betulaceae pollen grains. Average percentages between 1.5-3% were reached by *Ostrya/Carpinus orientalis*, *Celtis*, *Ulmus*, *Platanus*, *Olea* and other Oleaceae.

NAP (average value 26%, APF 19,000 grains/gram) reached the highest value (53%, APF 25,000 grains/gram) at the site 6 and the lowest value (11%, APF 2,500 grains/gram) at the site 3.

3.2. Land cover analysis

The land cover in the buffers surrounding the sites is reported in Figure 3A (for detailed values, see SI1 in supplementary file). The land cover percentages (Figure 3B) evidenced the prevalence of the built-up areas (average value close to 75%). In the 125m buffers, built-up area reached the highest value at the sites 9 and 14 (about 99.5%) and the lowest value at site 7 (34.4%). In the 250m buffers, this category reached the highest value at the site 9 (98%) and the lowest at the site 2 (about 43%). In the 500m buffers, urbanized areas showed the highest value at the sampling site 15 (96%), the lowest at the sampling site 2 (about 45%).

Arboreal coverage showed an average percentage of about 11-12% in the 125m and 250m buffers, and more than 15% in the 500m buffers: the highest arboreal coverage is showed at the site 4 (respectively 36%, 40%, 35%) and the lowest at the site 9 (respectively 0.4%, 0.3%, and about 3%).

Herbaceous coverage showed an average percentage of 4-5%. The highest value was showed at the sampling site 4 (respectively 15%, 13%, and 12%), the lowest at the site 9 (respectively absent, 1%, and 1.6%).

The sites close to the water (Arno river) showed an average percentage of about 6-8% in the different radius buffers.

3.3. Tree point analysis

In the studied area the total number of trees is more than 12,000. The results concerning the spatial distribution of trees (SI2) showed that the highest concentration was at the sampling site 2, the lowest at the sampling site 9, where the 125 and 250m buffers did not show trees. The most represented tree was *Olea* (ca. 2,600 plants in total). The second most frequent trees were Cupressaceae/Taxaceae (ca. 2,300 plants); the third one *Quercus* (ca. 2,100 plants). The highest number of *Quercus* was observed around the sampling site 2. About 1,500 trees belonged to *Tilia* and 1,200 to *Celtis* L. The highest number of *Tilia* trees was recorded at the sampling site 3 for the 125m and 250m buffers and

at the sampling site 1 for the 500m buffers. The highest number of *Celtis* trees was at the site 4 for the 125m and 250m buffers and 2 for the 500m buffers.

3.4. Statistical analysis

3.4.1. Land cover analysis

Statistical model correlating APF of AP with the tree covered areas (arboreal coverage, Figures 2 and 3) in the three different lengths buffers showed no correlation for the 125m buffers (*p-value* 0.1) (Table 2). A slight correlation was shown for the 250m buffers (*p-value* 0.07, *Adj Rsq* 0.08); greater correlation for the 500m buffers (*p-value* 0.02, *Adj Rsq* 0.15).

No correlation was observed between APF of NAP and the areas covered by meadows in the 125m buffers (*p-value* 0.1). The correlation is very high for the 250m buffers (*p-value* 0.0001, *Adj Rsq* 0.30); it slightly decreases for the 500m buffers (*p-value* 0.001, *Adj Rsq* 0.25).

3.4.2. Tree point analysis

The results of the statistical analysis correlating the APF of each arboreal plant with the number of the corresponding source-plants found in each buffer are shown in Table II.

In the 125m buffers, the strongest statistical correlation was shown by *Celtis* (*p-value* 0.04, *Adj Rsq* 0.10), *Quercus* (*p-value* 0.04, *Adj Rsq* 0.09) and *Tilia* (*p-value* 0.005, *Adj Rsq* 0.20). In the 250m buffers the highest statistical correlation was shown by *Celtis* (*p-value* 0.05, *Adj Rsq* 0.09), *Quercus* (*p-value* 0.05, *Adj Rsq* 0.09) and *Tilia* (*p-value* 0.008, *Adj Rsq* 0.17). In the 500m buffers, the highest correlation was shown by Cupressaceae/Taxaceae (*p-value* 0.04, *Adj Rsq* 0.08), Juglandaceae (*p-value* 0.08, *Adj Rsq* 0.07), *Platanus* (*p-value* 0.08, *Adj Rsq* 0.06) and *Quercus* (*p-value* 0.02, *Adj Rsq* 0.14).

Regarding the graphical representation of the PCA, the first two axes are responsible for 99.9% of the total variance, respectively 98.5% and 1.4%. Vectors show that the large part of the variance is explained by Poaceae, Urticaceae and Asteraceae (Figure 4).

4. Discussion

The analysis of the moss cushions allowed to identify the pollen grains which reach the Florence city center at the height where people walk. The analysis performed at different sampling sites reveals an inhomogeneous pollen accumulation due to the contribution of numerous factors. The distribution of the urban green areas is one of the main factor; its influence on the pollen amount is measured by means of the land cover analysis. The results show that APFs have a mostly linear correlation with the extension of the green areas moving away from the sampling point, reaching the highest value in the 500m buffer (Table II). Low pollen values were found in sampling sites corresponding to large squares (sites 5, 9, 15) without ornamental trees and surrounded by extensive built-up areas (Figures 3 and SI). The highest values were generally recorded in the southern part of the city center (Figure 2).

From a qualitative point of view, all the samples display a greater amount of AP than NAP, with values similar to those which commonly occur in sites covered by woods (Ruffaldi 1994). A general prevalence of AP was also observed in, other Italian towns (Perugia, Romano et al. 1988; Genoa, Guido et al. 1992). In this study, AP reaches highest values in proximity of the green areas (Figure 5A), underlying the importance of the local presence of trees.

The spatial analysis *taxon* by *taxon* shows the different ways of pollen diffusion of the plants.

Cupressaceae/Taxaceae, widespread both in urban and extra-urban areas, are the most represented *taxon* in both pollen datasets in spite to their general under-representation in pollen spectra with respect to other plants (Ciani et al. 2019). This is due to the breakage of these grains during hydration (Duhoux 1982; Danti et al. 2011), which reduces their preservation. The proximity of cypresses has a significant effect on the pollen records, and can explain the majority of the local variations inside Florence (Ciani et al. 2019). The relation between their AP values and the coverage by these plants reaches the highest correlation in the 250m buffers (Table II).

Cupressaceae/Taxaceae average pollen percentages show a general decrease from 2008 to 2017 (from 22% to 15%), a trend which possibly has started few years before, according to studies in Emilia

Romagna, Italy (Mercuri et al. 2013). The local presence of the plants (proximity effect) is testified by the dramatic decline of Cupressaceae pollen at sites 12 (San Lorenzo, Figure 2) where two tall cypresses were cut in 2012.

Quercus, both deciduous and evergreen species, is the second most represented *taxon* in all the samples. These trees are numerous in Florence, a selection that reflects the aesthetic preference for ornamental trees, as in many other towns (Staffolani et al. 2011; Sallustio et al. 2017). *Quercus* AP values and tree coverage shows a good correlation in all the three buffers: the relation reached the highest significance in the 500m buffer and the lower in the 250m buffer. Although medium-long range distance transport of *Quercus* pollen is well-known (Maya-Manzano et al. 2016), the presence of a high number of oaks in the nearby and the considerable specific gravity of the pollen grains explains the high local pollen amount (Bryant et al. 1989; Pluess et al. 2009). The proximity effect of *Quercus* is evident at the site 3, *Piazza della Libertà*, (50% in 2008 and about 54% in 2017) where *Q. ilex* trees are very numerous (SI2). As a whole, *Quercus* pollen values increase from 14% in 2008 to 21% in 2017 (Figure 2).

The amounts of Pinaceae pollen, the third most represented *taxon*, have not statistical correlation with the presence of the trees in the surroundings (Table II). This is possibly due to the long-distance transport of the grains (Szczepanek et al. 2017) that reduces the effect of proximity to the source-plants. The average value of Pinaceae pollen increases from 6% in 2008 to 13% in 2017 (Figure 2).

Celtis and *Tilia*, two entomophilous trees, show a strong proximity effect (Table II, Figure 5C and 5D). Both these plants display a decrease of the statistical significance between the pollen values and the number of plants when the buffer areas increase: the highest correlation is showed in the 125m buffer and is absent in the 500m buffer.

Celtis, a tree with a high resistance to stress conditions (Brunetti et al. 2019), is widely used as ornamental tree on the roadsides in many part of the city, in particular in the south-western area of the city center. The amount of pollen grains is directly related to the position of the source-plants (Gassmann and Pérez 2006), as confirmed by the concentration map (Figure 5C).

Tilia represents about the 15% of the total road trees, the most used plants of urban green areas in Florence (Table I). Despite its low pollen production (Li et al. 2015), *Tilia* is an important allergenic tree which presents a prolonged flowering periods inside the town: studies on the effects of the urban environments on the phenology of *Tilia* demonstrated a direct influence of the density of built-up surfaces, while no relation was found with the building volume density (Massetti et al. 2015).

Juglandaceae and *Platanus* pollen values display a slight statistical correlation in the 500m buffers. *Platanus* is very common along the main roads of the city (Table I); its short but intense pollen season can lead to high local pollen concentrations (Lara et al. 2019), as shown in site 11 in the 2008 and in site 10 in 2017. The local influence of these trees to the pollen records was already observed by Bricchi et al. (2000) and Manzano et al. (2017).

Great interest has the pollen data recorded at the sites that are devoid of trees and located in a 500m buffer with dominance of built-up surfaces. This is the case of the Cathedral square (site 15 *Santa Maria del Fiore*, Figures 2 and 3). The moss samples showed a prevalence of AP pollen (more than 75%) in both 2008 and 2017. The lack of any tree at the site and the scarce arboreal cover in the nearby hints the possible pollen transport from distances longer than the 500m buffer, perhaps from the tree cover of the surroundings of Florence, rather than from plants inside the city, because of the shelter due to the buildings. This hypothesis is supported by the similar pollen spectra of site 15 and other large squares (site 5, 9) in 2008 and 2017. The data are also in agreement with the ARPAT (Regional Agency for environmental protection of Tuscany) records, made by means of a volumetric trap which well reflects the pollen contribution from outside of the city over the roof (Ciani et al. 2019).

Regarding NAP, the statistical model reveals that the highest correlation with the herbaceous land cover is reached in the 250m buffer: the correlation is not significant in the 125m buffer, while it decreases in the 500m buffer (Table II). Therefore, herbaceous land cover seems to contribute to the pollen recorded more in a shorter radius than the arboreal plants.

In Florence, the average NAP percentage decreases from 33% in 2008 to 26% in 2017. This trend of the herbaceous plant pollen has been observed in others Mediterranean areas and attributed to the substantial decrease in grasslands (D'Amato et al. 2007). NAP values show stability over the years at the site 6 (*Ponte Amerigo Vespucci*) where the highest NAP percentages are recorded in both sampling years. The same trend is observed also in the sites 7 (*Ponte alle Grazie*) and 2 (*Porta San Niccolò*). These sites where NAP show the highest concentrations are located in the southeastern part of the city (Figure 5B), close to the Arno River: here ruderal and nitrophilous herbs are widespread along the river banks (Foggi et al. 2008).

The most statistically significant NAP belongs to Poaceae and Urticaceae (Figure 4), two of the most frequent families in the pollen spectra (Spiekma et al. 1989; De La Guardia et al. 1998) including the highest allergenic potential herbs (D'Amato and Lobefalo 1989). These aspects, combined with the strictly local pollen contribution of herbaceous plants, suggest the necessity to follow precise policies for meadows cutting, in addition to a constant management of urban streets.

5. Conclusions

Despite moss samples do not provide an accurate temporal registration, they represent a useful and economic tool for obtaining general surveys and reliable record of the pollen diffusion and accumulation. Their analysis evidenced that most part of the pollen grains which reach the ground level in Florence belong to arboreal plants. This is mainly due to Cupressaceae and *Quercus*, that are widespread inside the city and in extra-urban areas and diffuse their pollen grains at great distance. Other plants, like *Celtis* and *Tilia*, commonly used as ornamentals trees along the main roads, show a strictly local pollen diffusion.

Herbaceous plants diffuse pollen at relatively short-distance, Poaceae and Urticaceae being the most influent on pollen concentrations.

The pollen accumulation is uneven across the city. In open spaces surrounded by built-up areas, the moss samples record AP assemblages which possibly reflect the pollen contribution from outside of

the city. Near the urban green areas, the pollen values are strongly affected by the presence of the dominant trees.

This study confirms the importance of an accurate selection of the trees to be planted in the urban contexts and the necessity of an accurate management of the green spaces.

Figure 1: Geographical location of Florence (A); the sampling area (B) and the list of the sites.



Figure 2: Results of the pollen analysis made on 2008 samples (grey columns) and 2017 samples (black columns). Pollen values are expressed as percentages (A), and APF (pollen grains/grams*100) (B).

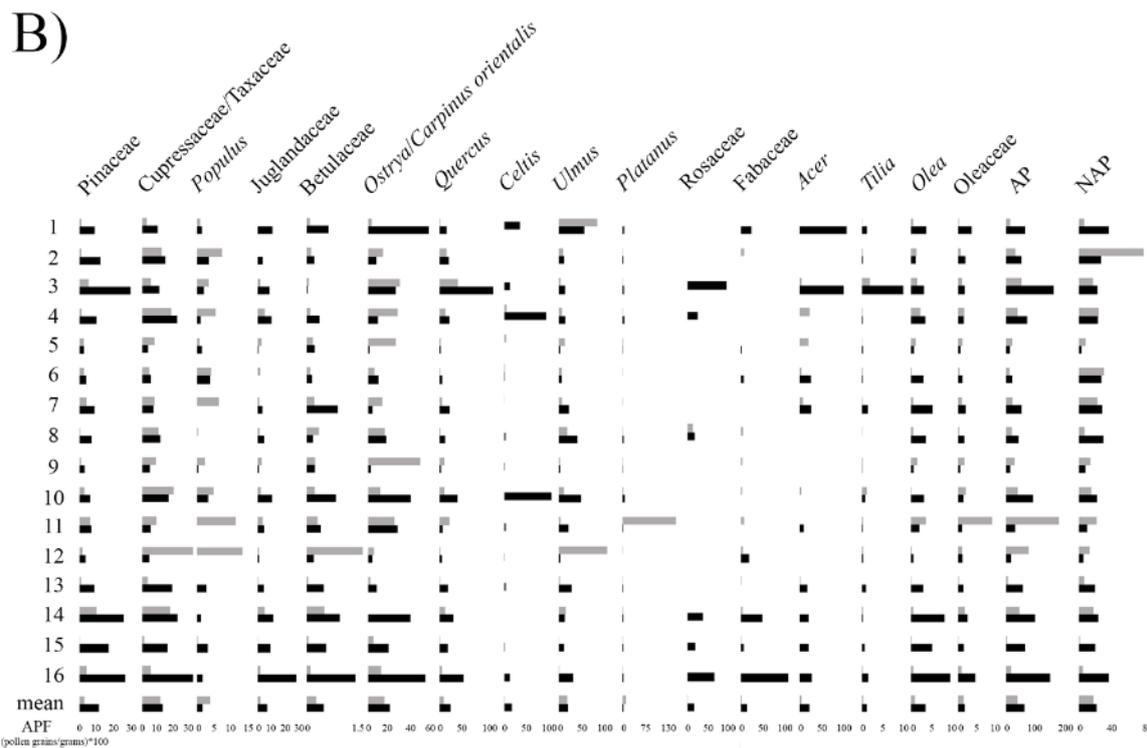
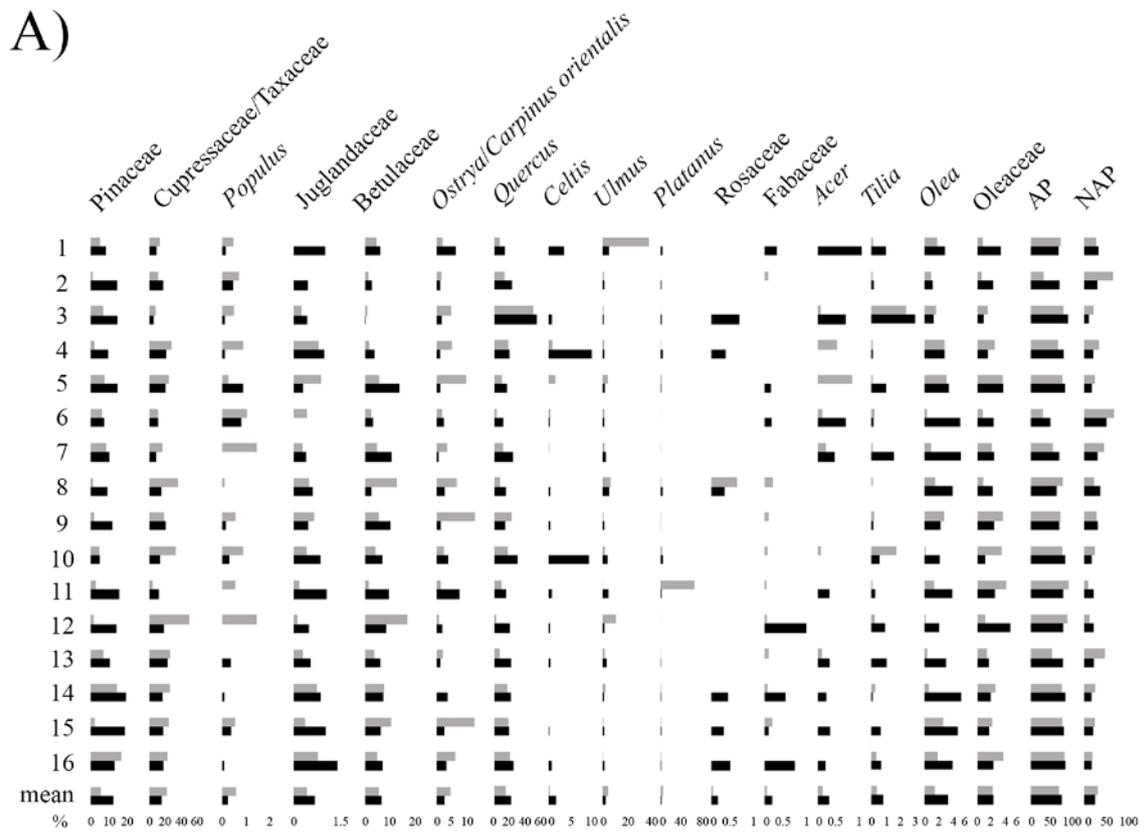


Figure 3: Land cover map of the sampling area (A) and percentages of the land cover categories surrounding the sampling points in the buffers (B).

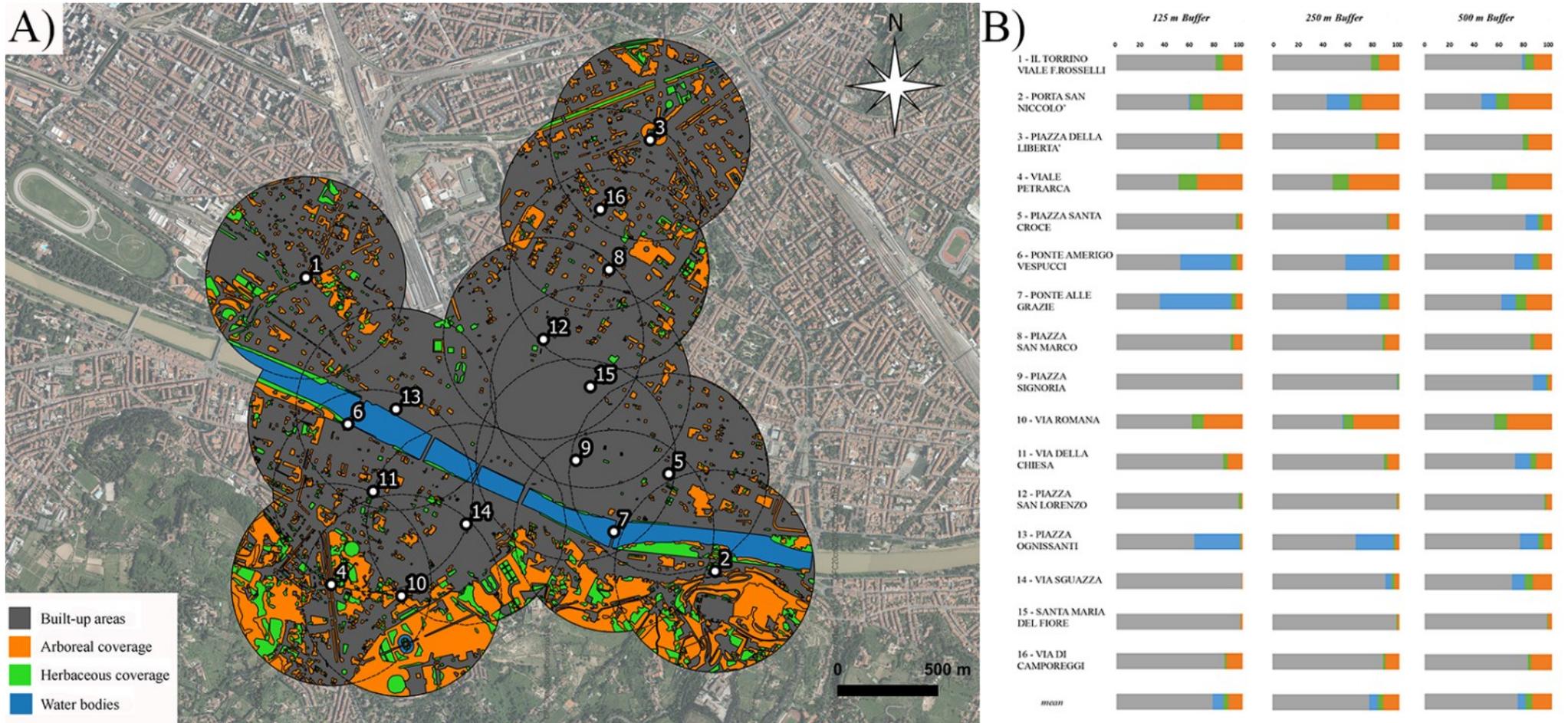


Figure 4: Graphical representation of PCA for NAP concentrations.

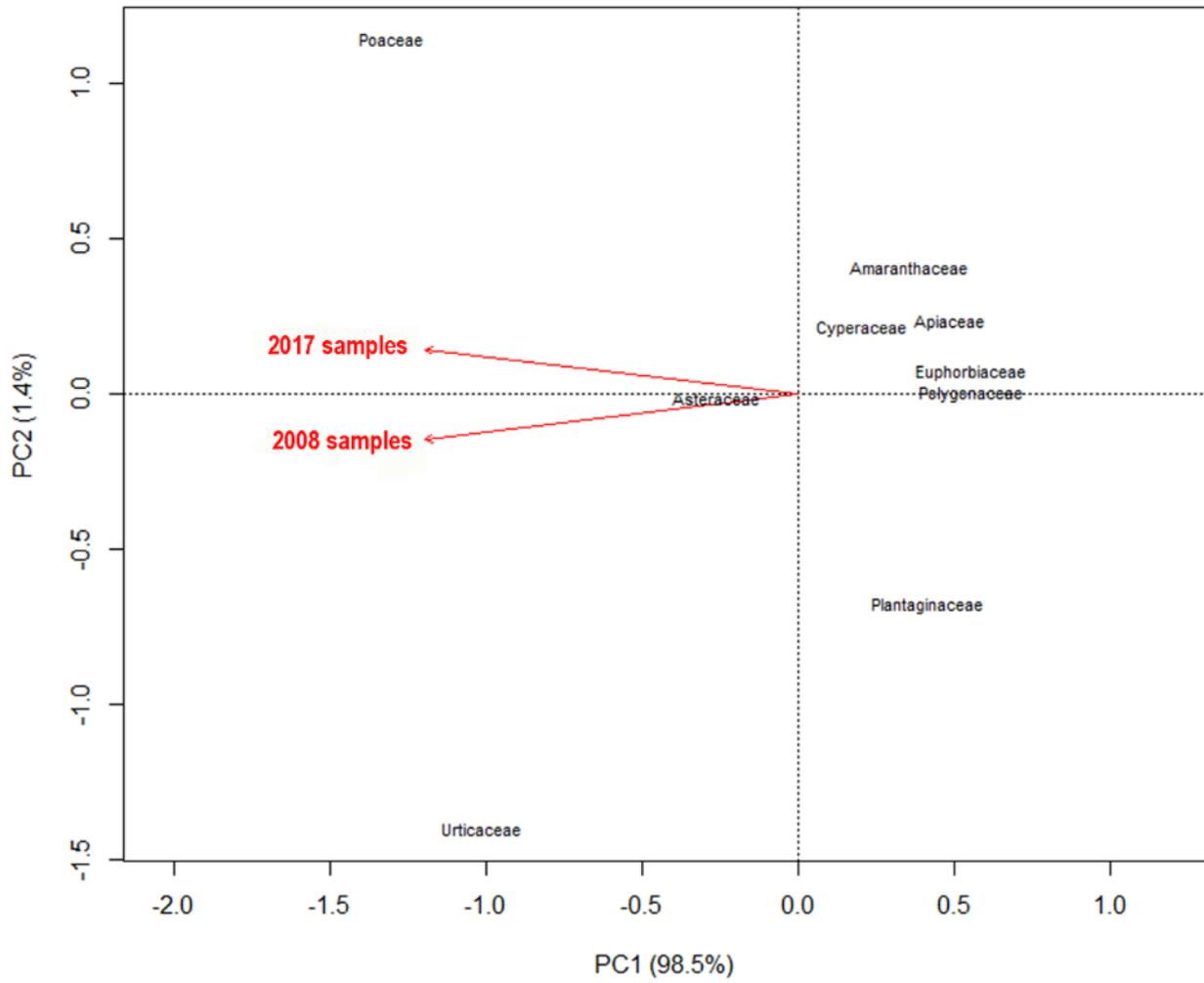


Figure 5: Pollen concentration maps drawn using inverse-distance weighting spatial interpolation. AP (A); NAP (B); *Celtis* (C); *Tilia* (D).

Yellow/green points in figure (C) and (D) refer respectively to *Celtis*/*Tilia* trees.

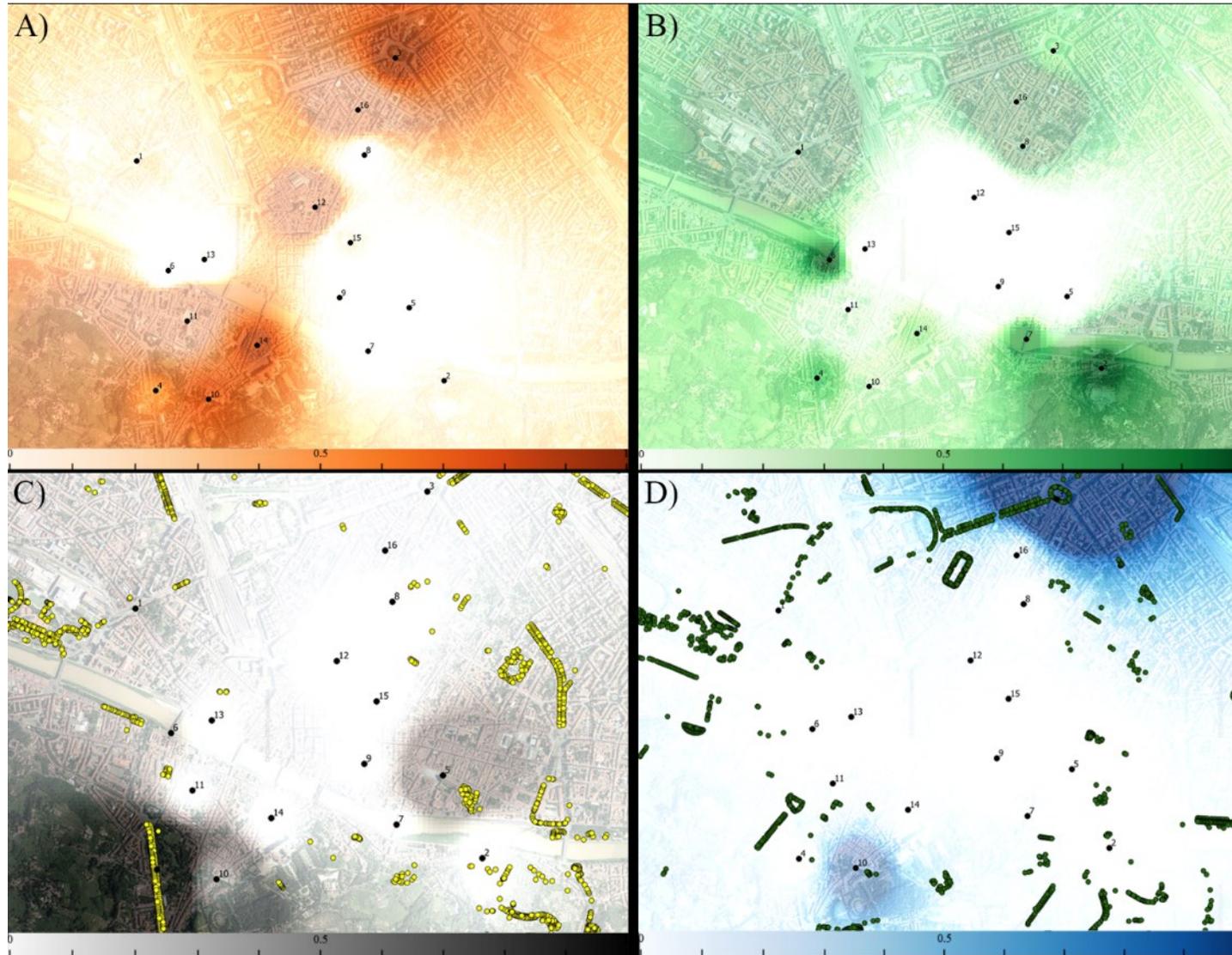


Table I: The most used trees of urban green areas in the municipality of Florence.

	<i>Number of trees</i>	<i>Percentage on the total number of trees</i>
<i>Tilia</i>	8426	15
Cupressaceae/Taxaceae	7945	14
<i>Quercus</i>	7872	14
<i>Celtis</i>	6757	12
<i>Pinus</i>	4481	8
<i>Platanus</i>	4268	7
<i>Ulmus</i>	3353	6
<i>Acer</i>	3323	6
<i>Olea</i>	3586	6
<i>Populus</i>	2226	4
<i>Fraxinus</i>	2531	4
<i>Cedrus</i>	1360	2
Others	1348	2

Table II: Statistical results of the repeated-measures ANOVA for Land cover analysis and Tree point analysis. The extensions of arboreal/herbaceous coverage of each buffer area of different length is expressed in m2. Significance levels: p-value< 0.0001 ***; p-value< 0.001 **; p-value< 0.01 *; p-value< 0.05 .

	Land cover analysis											
	Buffer 125 m				Buffer 250 m				Buffer 500 m			
	<i>SS</i>	<i>F</i>	<i>p-value</i>	<i>Adj Rsq</i>	<i>SS</i>	<i>F</i>	<i>p-value</i>	<i>Adj Rsq</i>	<i>SS</i>	<i>F</i>	<i>p-value</i>	<i>Adj Rsq</i>
APF total ~ Total green areas	2.41	3.56	0.04*	0.13	2.78	4.19	0.03*	0.16	4.49	7.41	0.02*	0.22
APF AP ~ Arboreal coverage	-	-	-	-	2.57	3.12	0.07	0.08	4.62	6.13	0.02*	0.15
APF NAP ~ Herbaceous coverage	-	-	-	-	4.52	8.73	0.0001***	0.30	4.01	7.47	0.001**	0.25
	Tree point analysis											
APF <i>Celtis</i>	839	4.35	0.04*	0.10	3352	4.04	0.05	0.09	-	-	-	-
APF Cupressaceae/Taxaceae	-	-	-	-	54048	4.43	0.01*	0.10	-	-	-	-
APF Juglandaceae	-	-	-	-	-	-	-	-	18.71	3.26	0.08	0.07
APF <i>Platanus</i>	-	-	-	-	-	-	-	-	10096	3.22	0.08	0.06
APF <i>Quercus</i>	2440	4.30	0.04*	0.09	11235	4.03	0.05	0.09	149111	5.77	0.02*	0.14
APF <i>Tilia</i>	1725	8.89	0.005**	0.20	3274	7.95	0.008**	0.17	-	-	-	-

S11: Results of the land cover analysis. The extensions of the land cover categories are reported as m2 and percentage. 125 m buffers:

		Built-up areas	Water bodies	Herbaceous coverage	Arboreal coverage
1 - Il Torrino Viale F.Rosselli	m2	38464	0	2919	7531
	%	78.6	0.0	6.0	15.4
2 - Porta San Niccolò	m2	28035	679	4932	15269
	%	57.3	1.4	10.1	31.2
3 - Piazza della Libertà	m2	38932	307	1133	8542
	%	79.6	0.6	2.3	17.5
4 - Viale Petrarca	m2	24111	0	7229	17574
	%	49.3	0.0	14.8	35.9
5 - Piazza Santa Croce	m2	46293	0	1028	1593
	%	94.6	0.0	2.1	3.3
6 - Ponte Amerigo Vespucci	m2	24715	19749	2250	2199
	%	50.5	40.4	4.6	4.5
7 - Ponte alle Grazie	m2	16842	27707	1772	2592
	%	34.4	56.6	3.6	5.3
8 - Piazza San Marco	m2	44315	0	1087	3513
	%	90.6	0.0	2.2	7.2
9 - Piazza Signoria	m2	48709	0	0	204
	%	99.6	0.0	0.0	0.4
10 - Via Romana	m2	29303	0	4529	15082
	%	59.9	0.0	9.3	30.8
11 - Via della Chiesa	m2	41336	0	1779	5799
	%	84.5	0.0	3.6	11.9
12 - Piazza San Lorenzo	m2	47309	0	1198	407
	%	96.7	0.0	2.4	0.8
13 - Piazza Ognissanti	m2	30152	17704	444	614
	%	61.6	36.2	0.9	1.3
14 - Via Sguazza	m2	48664	0	15	235
	%	99.5	0.0	0.0	0.5
15 - Santa Maria del Fiore	m2	48029	0	140	745
	%	98.2	0.0	0.3	1.5
16 - Via di Camporeggi	m2	41951	0	682	6282
	%	85.8	0.0	1.4	12.8
<i>mean</i>	m2	37323	4134	1946	5511
	%	76.3	8.5	4.0	11.2

S11 (continued). 250 m buffers:

		Built-up areas	Water bodies	Herbaceous coverage	Arboreal coverage
1 - Il Torrino Viale F.Rosselli	m2	150178	0	12107	30961
	%	77.7	0.0	6.3	16.0
2 - Porta San Niccolò	m2	82639	34492	19151	56964
	%	42.8	17.8	9.9	29.5
3 - Piazza della Libertà	m2	156767	309	3940	32231
	%	81.1	0.2	2.0	16.7
4 - Viale Petrarca	m2	91436	0	24825	76985
	%	47.3	0.0	12.8	39.8
5 - Piazza Santa Croce	m2	173960	0	2823	16463
	%	90.0	0.0	1.5	8.5
6 - Ponte Amerigo Vespucci	m2	111152	57580	9064	15450
	%	57.5	29.8	4.7	8.0
7 - Ponte alle Grazie	m2	113025	51168	12749	16304
	%	58.5	26.5	6.6	8.4
8 - Piazza San Marco	m2	167591	0	3934	21721
	%	86.7	0.0	2.0	11.2
9 - Piazza Signoria	m2	189429	1177	2015	625
	%	98.0	0.6	1.0	0.3
10 - Via Romana	m2	106597	1872	14944	69833
	%	55.2	1.0	7.7	36.1
11 - Via della Chiesa	m2	169845	237	5199	17965
	%	87.9	0.1	2.7	9.3
12 - Piazza San Lorenzo	m2	189004	0	1719	2522
	%	97.8	0.0	0.9	1.3
13 - Piazza Ognissanti	m2	126605	57637	2829	6174
	%	65.5	29.8	1.5	3.2
14 - Via Sguazza	m2	172041	9536	4478	7192
	%	89.0	4.9	2.3	3.7
15 - Santa Maria del Fiore	m2	188732	0	1728	2786
	%	97.7	0.0	0.9	1.4
16 - Via di Camporeggi	m2	168269	0	3645	21332
	%	87.1	0.0	1.9	11.0
<i>mean</i>	m2	147329	13375	7822	24719
	%	76.2	7.0	4.0	12.8

SI1 (continued). 500 m buffers:

		Built-up areas	Water bodies	Herbaceous coverage	Arboreal coverage
1 - Il Torrino Viale F.Rosselli	m2	601320	17094	55215	111998
	%	76.5	2.2	7.0	14.3
2 - Porta San Niccolò	m2	350547	91315	76408	267356
	%	44.6	11.6	9.7	34.0
3 - Piazza della Libertà	m2	605121	1074	33497	145936
	%	77.0	0.1	4.3	18.6
4 - Viale Petrarca	m2	413696	2562	91386	277983
	%	52.7	0.3	11.6	35.4
5 - Piazza Santa Croce	m2	623663	75580	29267	57118
	%	79.4	9.6	3.7	7.3
6 - Ponte Amerigo Vespucci	m2	553945	115162	35977	80544
	%	70.5	14.7	4.6	10.3
7 - Ponte alle Grazie	m2	473094	88709	65209	158617
	%	60.2	11.3	8.3	20.2
8 - Piazza San Marco	m2	652783	0	22788	110058
	%	83.1	0.0	2.9	14.0
9 - Piazza Signoria	m2	668883	82232	12462	22052
	%	85.1	10.5	1.6	2.8
10 - Via Romana	m2	428557	3454	75892	277725
	%	54.5	0.4	9.7	35.4
11 - Via della Chiesa	m2	557573	93494	37960	96600
	%	71.0	11.9	4.8	12.3
12 - Piazza San Lorenzo	m2	738938	0	12736	33954
	%	94.1	0.0	1.6	4.3
13 - Piazza Ognissanti	m2	588390	114526	31385	51326
	%	74.9	14.6	4.0	6.5
14 - Via Sguazza	m2	539355	79300	48890	118083
	%	68.7	10.1	6.2	15.0
15 - Santa Maria del Fiore	m2	755070	0	6889	23669
	%	96.1	0.0	0.9	3.0
16 - Via di Camporeggi	m2	636723	306	19417	129183
	%	81.0	0.0	2.5	16.4
<i>mean</i>	m2	574229	47801	40961	122638
	%	73.1	6.1	5.2	15.6

SI2: Results of the tree point analysis.

Buffer 125 m

	Sampling sites																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
<i>Acer</i> sp.											2						2
Betulaceae																	0
<i>Celtis</i> sp.	2	5	2	59													68
Cupressaceae/ Taxaceae	5	41		19	25	4		6		6	12		1		3	4	126
Fabaceae		1															1
Juglandaceae				3													3
<i>Olea</i> sp.																	0
Oleaceae		1															1
<i>Ostrya</i> / <i>Carpinus</i> o.	1																1
Pinaceae	7	15	5	3			6										36
<i>Platanus</i> sp.		4	3	14		1				3							25
<i>Populus</i> sp.		4															4
<i>Quercus</i> sp.		97	41	15			1			4	1						2 161
Rosaceae		2															2
<i>Tilia</i> sp.	10	6	64	3						8	2						93
<i>Ulmus</i> sp.	1	1	2					7									11
Total	26	177	117	116	25	5	7	13	0	21	17	0	1	0	3	6	534

Buffer 250 m

	Sampling sites																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
<i>Acer</i> sp.		11		3	6	6	1	2			2				2		33
Betulaceae		5	2		1		1	4									13
<i>Celtis</i> sp.	6	15	40	114	61	22	9	5			13		4				289
Cupressaceae/ Taxaceae	23	94	5	121	32	52	10	22		35	25	1	7	5	4	13	449
Fabaceae		29		4	3	2	12	4									54
Juglandaceae		1		5	1	1		4								1	13
<i>Olea</i> sp.		230			2			2							16		250
Oleaceae	20	3		1	4	8	1	4			2						43
<i>Ostrya</i> / <i>Carpinus</i> o.	1							1									2
Pinaceae	35	35	5	10	7	4	7	5		4					4	1	117
<i>Platanus</i> sp.	8	6	43	26	2	19		1		16	21						142
<i>Populus</i> sp.	26	4		2		3		1									36
<i>Quercus</i> sp.		205	47	73	12	9	3	25		114	11		4	12		4	519
Rosaceae	2	24		3	17	19		7			5	2				1	80
<i>Tilia</i> sp.	22	11	94	25	30	1	12	2		10	22			3			232
<i>Ulmus</i> sp.	1	19	3	2	4	3		10						11			53
Total	144	692	239	389	182	149	56	99	0	179	101	3	15	31	26	20	2325

Buffer 500 m

	Sampling sites																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
<i>Acer</i> sp.		33	17	11	10	35	17	2	2	8	38	2	11	2	2	2	192
Betulaceae		14	3		2		2	5									31
<i>Celtis</i> sp.	75	223	120	175	69	49	99	22	13	208	75	8	33	9	8	12	1198
Cupressaceae/ Taxaceae	53	503	64	269	50	149	181	97	36	220	186	54	128	130	20	88	2228
Fabaceae	4	130	2	4	13	6	33	4		11	6		6	1		7	227
Juglandaceae	1	2		6	1	1	1	4		6	7		1			5	35
<i>Olea</i> sp.		1417		596	19		489	2	16			8			16	3	2566
Oleaceae	103	14	7	5	4	51	20	4	1	5	11		26			5	256
<i>Ostrya</i> / <i>Carpinus</i> o.	1							1								1	3
Pinaceae	70	90	43	31	22	22	24	33	11	35	24	13	9	11	6	29	473
<i>Platanus</i> sp.	21	25	183	116	10	89	3	4		134	90		25	18	1	87	806
<i>Populus</i> sp.	26	10	4	2	4	3		1		2	2		3			1	58
<i>Quercus</i> sp.	23	303	88	213	20	42	79	97	3	660	119	8	13	331	1	120	2120
Rosaceae	6	117	17	8	18	37	44	12		7	22	3	23	2	6	8	330
<i>Tilia</i> sp.	192	172	160	118	67	52	50	121		123	81	27	13	33	2	315	1526
<i>Ulmus</i> sp.	19	109	4	12	8	10	26	12		48	15	7	4	11		13	298
Total	594	3162	712	1566	317	546	1068	421	82	1467	676	130	295	548	62	701	12347



5. Cupressaceae pollen: new data about diffusion, record and preservation

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Abstract

Cupressaceae is one of the most widespread families in the Mediterranean region, widely used as ornamental trees both in cities and in extra urban areas. The family is also known to produce a considerable amount of pollen grains. Nevertheless, Cupressaceae pollen is generally scarce in sediments and it is attested to be one of the most underrepresented pollen in ancient spectra. Cupressaceae pollen diffusion was detected by means of two sampling methods: moss cushions, which are able to accumulate pollen grains for several successive years, and volumetric sampler, which records the airborne pollen day by day. The study was conducted in the city of Florence (Italy), repeating moss samplings at the same sites for three times. The comparison between the two sampling methods revealed the low preservation of this pollen type and suggested that even low quantities of this pollen may indicate the local presence of the source plants. In order to test the contribution of the local pollen sources to the background of the airborne pollen, the pollen content of moss cushions was also related to the number of Cupressaceae plants surroundings each sampling site. The results indicate a noticeable influence of the plants growing in a short-range distance.

Keywords: Cupressaceae; pollen grains preservation; moss cushions; tree pollen contribution; repeated measures ANOVA.

Cover image: Cypress trees in the Boboli Gardens (Florence).

1. Introduction

Numerous studies have been devoted to the pollen diffusion of Cupressaceae, one of the most widespread plants in the Mediterranean area. These plants produce noticeable quantities of pollen grains which dominate the aerobiological record in urban contexts during winter/early spring. Moving from East to West in the Mediterranean basin, Cupressaceae pollen grains reached a maximum daily concentration of 1719 grains/m³ in Istanbul (Turkey) during March (Celenk et al. 2010); a maximum daily concentration of about 1543 grains/m³ was recorded in San Remo (Italy) during January (Ariano et al. 1994); peaks higher than 1000 grains/m³ (Díaz de la Guardia et al. 2006) were recorded in Granada (Spain) during February. In the last years, a further increase of Cupressaceae pollen grains has been observed, probably due to a change in meteorological conditions (Subiza et al. 2018) and climate warming (Mercuri et al. 2013). A noticeable part of the airborne Cupressaceae pollen belongs to *Cupressus* whose production has been calculated around 400,000 grains for each cone in *C. sempervirens*, as also *C. macrocarpa* and *Hesperocyparis glabra* (Sudw.) Bartel (= *C. arizonica*) (Hidalgo et al. 1999).

Pollen production and diffusion of the plants can also be studied through the analysis of moss cushions. Moss cushions are natural traps able to accumulate and preserve the pollen grains of several successive years, from two up to fifteen years (Crowder & Cuddy 1973; Bradshaw 1981; Caseldine 1981; Cundill 1991). According to a recent study, the most significant statistical correlation occurs when the total pollen content of the mosses is related to the total airborne pollen of five-eight years (Ciani et al. submitted). This span of time might represent the period after which a balance is reached between the accumulation of new pollen grains and the rate of degradation of the old ones.

Moss samples are widely used also as “modern analogues”, an informative tool to interpret fossil pollen spectra and to relate the pollen rain to the surrounding vegetation (Räsänen 2001; Graf & Chmura 2006).

In a study on the relationship between pollen diffusion and pollen deposition, Spiexsma et al. (1994) reported that the Cupressaceae pollen is under-represented in the moss cushions. Under-

representation may be also inferred by the record of scarce amount of Cupressaceae pollen in ancient sediments of areas - for example the Roman city of Pompeii, Italy (e.g. Jashemski 1993) - where the macroremains attest the past presence of numerous plants (Borgongino 2006). Even in the soil samples collected in the Garden of Casti Amanti (Insula XII, Regio IX, Pompeii), where six *Juniperus* plants grew in a small space (about 110 m²), Cupressaceae pollen was no more than 3.5% of the total record (Ciarallo & Mariotti Lippi 1993).

Cupressaceae are commonly used as ornamental trees in many Mediterranean towns and, in particular, in Florence. They have a prolonged flowering period (Sabariego et al. 2012) and consequently a long-lasting presence of their pollen in atmosphere (Aira et al. 2011). This behavior makes Cupressaceae pollen one of the main causes of winter allergic respiratory diseases (Díaz de la Guardia et al. 2006). Moreover, the numerous airborne orbicules detached from these grains seem to play an important role in enhancing pollinosis (Ruggiero & Bedini 2018). For this reason, the Cupressaceae pollen concentrations are daily monitored by the local health agencies.

The aim of this study was to compare the quantities of Cupressaceae pollen grains collected by the moss cushions with those recorded by a volumetric sampler, in order to clarify the relationship between these two types of records. The pollen content of moss cushions has been also related to the quantity of the Cupressaceae growing at short-distance from the sampling sites, in order to prove the contribution of the local sources to the pollen record.

2. Materials and Methods

2.1 Study area

Florence (43°47'14"64 N, 11°14'59"64 E, 50 m a.s.l.) is located in the north-eastern part of Tuscany, central Italy, in the middle of the Arno river basin (Figure 1A). The climate is sub-Mediterranean, with dry summers, wet autumns and springs and mild winters (Petralli et al. 2011). The average annual temperature is 15.5° C, while the annual average rainfall is around 830 mm (Ximenian Observatory of Florence 2005). The slopes of reliefs surrounding the city are covered by more or less

open woods alternate with private parks and olive tree. In the urbanized areas, spontaneous vegetation is represented by small remnant woody patches and meadows and by the riparian vegetation along the Arno river and its tributaries. Cupressaceae trees are widespread in Florence for ornamental purpose: they are the second most used plant in the urban green areas and its number has increased in the recent years (Municipality of Florence, Territorial Information System 2010, 2016). Cypress trees also grow in the surrounding, mainly along roads and driveways, near villas, churches, chapels and cemeteries.

2.2 Moss sampling and pollen analysis

Moss cushions were collected in 16 sites (Figure 1B) inside the historical town center of Florence in 1995, 2008 and 2017, in order to study the relationship between pollen rain and vegetation.

The 48 samples were treated using routine methodologies followed by acetolysis (Erdtman 1960). Pollen analysis was carried out at light microscopy, operating at 400-630 \times . Absolute Pollen Frequency (APF) was calculated as the number of grains per gram of sample. Pollen percentages were calculated on total grains. Some pollen grains of Taxaceae (*Juniperus* type according to Moore et al. 1991) may have been included in the Cupressaceae pollen sum.

Concentration maps of Cupressaceae APF were drawn using Quantum GIS software (Quantum G. I. S. Development Team 2013) in order to display the pollen diffusion inside the city of Florence.

2.3 Comparison between moss samples and volumetric sampler pollen records

The moss samples (MS) average percentages were compared to those of the volumetric sampler (VS) of the monitoring station of ARPAT (Regional Agencies for Environmental Protection of Tuscany) located above the roof of the main hospital of the city (AOU Careggi), few kilometers far from the city center (Fig. 1B). The records are available starting from January 2000 and may be freely downloaded from the Internet site of ARPAT (<http://www.arpat.toscana.it>) where sampling methods are also reported. The VS average percentages are referred to five, six, seven and eight years before

the two moss samplings. The period of five-eight years was chosen on the basis of a previous study (Ciani et al. submitted). Regarding the last sampling, the comparison was also performed between the MS and VS absolute pollen values, due to the availability of the VS data also reported as the number of grains per cubic meter.

2.4 Proximity effect of trees to the pollen records and statistical analysis

For evaluating the pollen diffusion at short distance and the local contribution to the total pollen rain, the amount of Cupressaceae pollen grains of the moss samples (1995, 2008 and 2017) was statistically compared to the number of Cupressaceae trees growing around the sampling sites. The trees were counted inside two buffer areas of 250 and 500 meters in radius drawn around each sampling site using Quantum GIS software. The APF of Cupressaceae was respectively correlated with the sampling sites and the amount of the plants included inside each buffer of different radius. Statistical correlations were made using general linear models and repeated-measures analysis of variance (ANOVA) over the three years (1995, 2008 and 2017) in each site. All the pollen data were log-transformed in order to normalize the data. The analysis was performed using R-Studio software (R Core Team 2018).

The data about the number and the distribution of the trees (Cupressaceae + Taxaceae) were provided by the Municipality of Florence (Territorial Information System 2010, 2016; <http://sit.comune.fi.it/servizi.asp>) and integrated with the use of orthophotos (Territorial and Environmental Information System of Tuscany; <http://www.regione.toscana.it/-/geoscopio>) acquired during the different years of moss sampling and by direct observations in the study area. Taxaceae trees are particularly abundant in the South-eastern part of the sampling area.

3. Results

3.1 Moss samples and volumetric sampler pollen records

The analysis of the moss cushions (Table I) revealed a quantity of Cupressaceae pollen ranging from 220 to 27,700 grains/gram, mean value 10,360 grains/gram, in 1995; from 1100 to 45,200

grains/gram, mean value 10,400 grains/gram, in 2008; from 2350 to 32,400 grains/gram, mean value 10,600 grains/gram, in 2017. Percentages varied from 7% to 43%, mean value 18%, in 1995; from 4% to 49.5%, mean value 21%, in 2008; from 4% to 17%, mean value 13%, in 2017.

The VS records (Table I) ranged from 44.5% (average of eight years) to 53% (average of six years) during the period 2000 - 2007 and from 44% (average of five years) to 52% (average of eight years) during the period 2009 - 2016. The absolute values (grains/m³) of the VS pollen record during 2009-2016 varied between 13,280 grains/m³ and 8508 grains/m³.

3.2 Proximity effect of Cupressaceae trees to the pollen records

The results about the number of trees placed inside the two different range buffers in each sampling site are reported in Table II. The sum of Cupressaceae trees inside all of the buffers of 250 m radius ranged from 347 trees in 1995 to 406 trees in 2008. Considering each buffers separately, the higher number of Cupressaceae trees resulted in the sampling site “Viale Petrarca”, about 100 trees. The lower number of trees was found in “Piazza della Signoria”.

The total number of Cupressaceae trees inside the 500 m radius buffers ranged from 1880 trees in 1995 to 1939 trees in 2008. The higher number was found in the sampling site “Porta San Niccolò”, more than 400 trees; the lower number in the sampling site “Santa Maria del Fiore”, about 20 trees. The results of the statistical analyses which relate Cupressaceae APF, the sampling sites and the numbers of trees inside different radius buffers are shown in Table III.

4. Discussion

The availability of numerous pollen records in Florence, both by a volumetric sampler and moss cushions collected in different sites and times, offers the opportunity to relate the air diffusion (VS record) and the deposition at the ground level of Cupressaceae pollen grains (MS record).

The results highlight that Cupressaceae pollen always displays higher values in the VS record than in the MS one. This result is in contrast with what was observed for the pollen of many other trees, for

example *Quercus*, the second most represented plants in the pollen records of Florence (Ciani et al. submitted). The different behavior of these pollen grains was already observed in previous studies (Spieksma et al. 1994; Lazarova et al. 2006; Lisitsyna et al. 2012).

It is to note that the MS pollen values are the record of the Cupressaceae pollen at the ground level, while the volumetric sampler is located at about 20 m a.g.l. (above ground level). Therefore, the lower values of MS compared to those of VS could be referred to the shielding effect of the urban buildings on the airborne pollen diffusion. In fact, urban settlements can reduce by some 20% the amount of pollen coming from outside the city (Gonzalo-Garijo et al. 2006). A more direct comparison between the MS and VS records would be furnished by the analysis of mosses collected at about the same height a.g.l. of the volumetric sampler. The analysis of several moss cushions sampled at 30 – 40 m a.g.l. in the city center of Florence revealed more or less the same percentages of Cupressaceae pollen (15-20% of the total) as in the moss samples collected at ground level (Mariotti Lippi & Mori Secci 1994; Begliomini 2000; Pampaloni 2017). Therefore, the height seems to have a scarce influence on the record of this pollen type by MS.

Given that the airborne Cupressaceae pollen is captured by both the traps, MS and VS, the lower amount in MS could be due to its scarce preservation. This may be related to the easy breakage of their fragile wall, which is thinner than 2 μm (Spieksma et al. 1994) rather than the degradation of the exine, which does not display a high susceptibility to corrosion (Havinga 1967). Indeed, the mechanism of hydration of the grains (Duhoux 1982; Danti et al. 2011) provokes the breakage of the exine layer: after the swelling of the intine, the thin exine breaks, wrinkles and folds on itself. Consequently, the preservation of these pollen grains is shorter than that of other pollen grains, e.g. *Quercus*. This is the presumable reason for the under-representation of Cupressaceae pollen in the moss cushions and even more in the sediments. In the study of the past pollen spectra for the reconstruction of ancient contexts, even low quantities of Cupressaceae pollen may indicate the local presence of these plants.

Considering the variation over the years, the VS data present overall stability. On the contrary, the average MS values of 2008 and 2017 present slight differences and the single MS, collected at different sites, shows marked changes in time, highlighting local differences. This result underlines the importance of recording the pollen concentration in several sites inside the urban contexts in order to determine the local actual risk factors for pollen allergic diseases, notably at the ground level, as MS do: the pollen content records well reflect the urban trees composition, as shown in concentration maps (Figure 2).

The statistical analysis between Cupressaceae APF from MS and the location and quantity of trees provides different results on the basis of the buffer area around the sampling sites.

In the 250 m buffers, the overall correlations between the Cupressaceae APF against the variables "sampling site" and "number of trees" are rather good (p -value = 0.02 and p -value = 0.01 respectively), with the amount of the trees that explained the 5% of the Cupressaceae APF variance of the moss samples.

In the 500 m buffer, the number of trees shows a lower level of significance (p -value > 0.05); the variable sampling sites is instead always important (p -value < 0.05).

Therefore, the Cupressaceae APF variance explained by the models may seem dependent by the number of tree only in the 250 m buffers and not in that of 500 m. It is possible that the scarce pollen preservation plays an important role in this result. We have also to consider that the model takes into account none of the meteorological parameters such as rainfall, air temperature relative humidity and wind. The first ones can be considered almost homogeneous in all of the city centers. Regarding the wind, despite being attested as one of the major factor affecting pollen air circulation (Silva-Palacios et al. 2000), its influence on cypress pollen seems to be rather weak (Damialis et al. 2005; Rojo et al. 2015).

Recent studies suggest that the majority of the airborne pollen reaching a place is coming from long distances (Oteros et al. 2015; Rojo et al. 2015). Nevertheless, this study confirms that the proximity of the pollen sources has a significant effect on the aerobiological spectrum, which can also explain

the majority of the local variations, as observed by Maya-Manzano et al. (2017). This seems to be particularly true in the case of Cupressaceae, as shown by previous studies carried out in Spain (Belmonte et al. 1999). Our research also assesses that the proximity of the Cupressaceae trees to pollen record becomes statistically more significant when a very small area is taken into consideration, e.g. the 250 m buffer rather than the 500 m one.

Figure 1: Study area. (A) Geographical location of Florence. (B) Sampling area and list of pollen record sites: volumetric sampler (VS) and moss cushions (numbers).

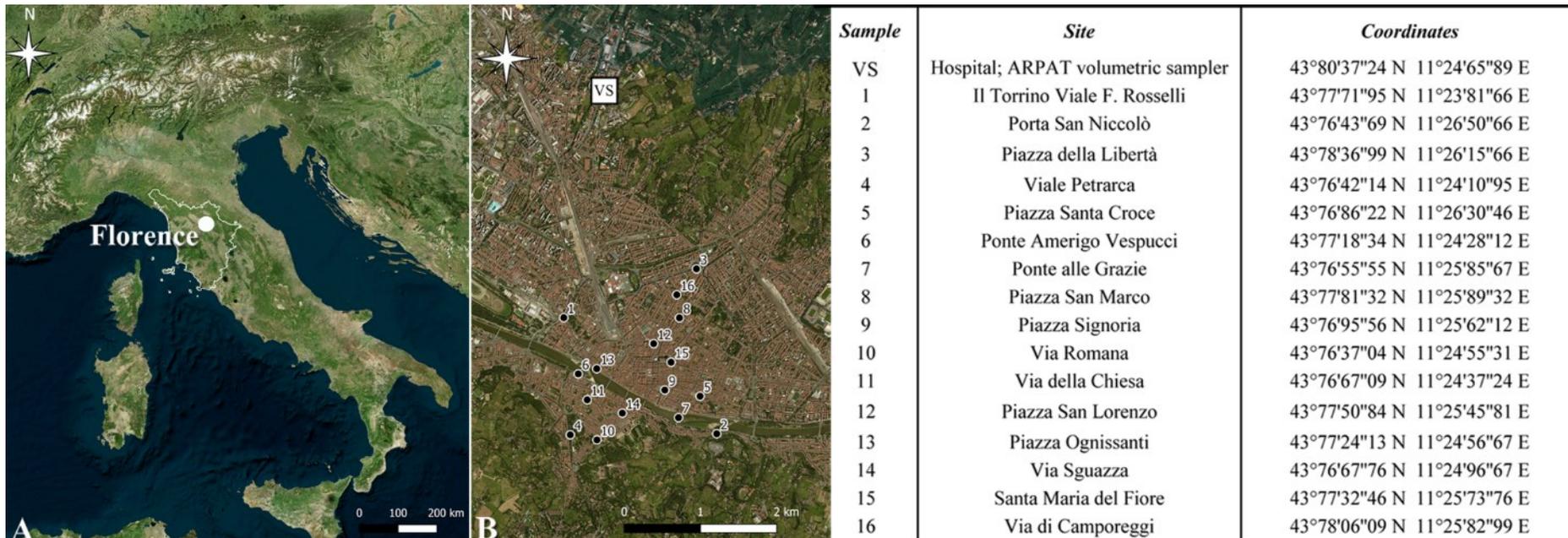


Figure 2: Cupressaceae APF concentration map recorded during the three years of sampling. The numbers refer to the moss cushions sampling sites; green dots are Cupressaceae and Taxaceae plants. Taxaceae are prevalent in the South-eastern part of the sampling area.

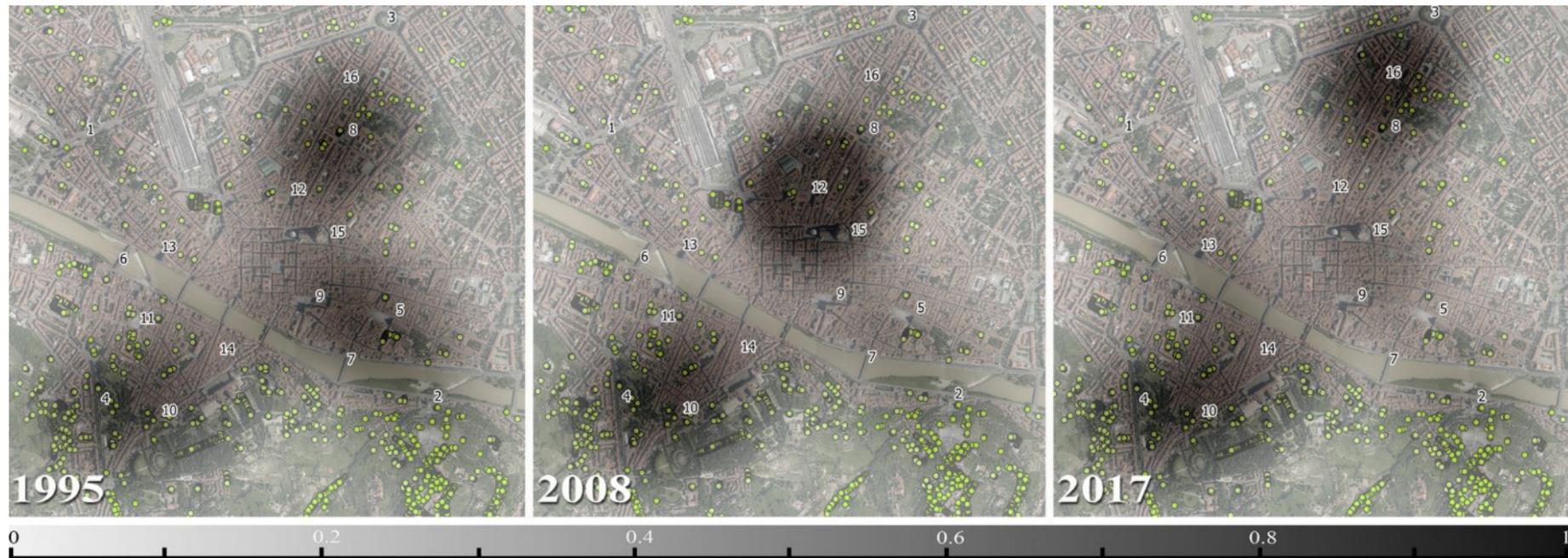


Table I: Cupressaceae pollen data recorded by moss cushions and volumetric sampler during the years studied.

Sample	Site	Moss cushions					
		1995		2008		2017	
		<i>APF</i>	%	<i>APF</i>	%	<i>APF</i>	%
1	Il Torrino Viale F. Rosselli	8190	7.7	2574	12.4	3889	6.8
2	Porta San Niccolò	13247	6.6	11083	10.6	13197	13.6
3	Piazza della Libertà	218	15.6	5083	7.3	9320	4.4
4	Viale Petrarca	19254	29.4	16510	26.5	19900	16.2
5	Piazza Santa Croce	19430	12.7	7075	22.9	3182	16.0
6	Ponte Amerigo Vespucci	1991	19.9	4127	10.4	2353	8.5
7	Ponte alle Grazie	4681	31.8	7060	15.8	3213	7.4
8	Piazza San Marco	27706	14.0	9200	34.6	5052	12.7
9	Piazza Signoria	17508	16.0	7698	17.0	4441	15.8
10	Via Romana	12323	12.8	17929	30.5	15053	11.6
11	Via della Chiesa	3228	25.2	8138	3.9	2396	11.9
12	Piazza San Lorenzo	9928	9.8	45226	49.6	4096	15.2
13	Piazza Ognissanti	6038	8.5	3076	24.4	17096	16.9
14	Via Sguazza	14938	42.7	16083	24.6	20114	15.8
15	Santa Maria del Fiore	2944	23.0	1103	22.9	14242	13.8
16	Via di Camporeggi	4082	6.7	4890	21.2	32381	16.7
	<i>mean</i>	10357	17.6	10428	20.9	10620	12.7

VS	Volumetric sampler			
	2000 - 2007		2009 - 2016	
	<i>grains/m³</i>	%	<i>grains/m³</i>	%
Average five years (2003 – 2007)	n.a.	51.7	Average five years (2012 – 2016)	13280 44.4
Average six years (2002 – 2007)	n.a.	53.2	Average six years (2011 – 2016)	11075 49.1
Average seven years (2001 – 2007)	n.a.	46.8	Average seven years (2010 – 2016)	9669 49.0
Average eight years (2000 – 2007)	n.a.	44.7	Average eight years (2009 – 2016)	8508 51.6

Table II: Cupressaceae/Taxaceae trees placed inside the two different range buffers (250 m and 500 m radius) in each sampling site during the three years of pollen sampling.

<i>Site</i>	Buffers 250 m			Buffers 500 m		
	1995	2008	2017	1995	2008	2017
	<i>Number of trees</i>			<i>Number of trees</i>		
Il Torrino Viale F. Rosselli	19	22	24	50	53	55
Ponte alle Grazie	14	15	15	172	173	173
Via della Chiesa	22	24	24	156	158	158
Piazza San Marco	15	19	18	53	57	56
Ponte Vespucci	10	48	32	107	145	129
Porta San Niccolò	64	67	48	450	453	434
Piazza della Libertà	5	5	5	62	62	62
Viale Petrarca	100	100	98	243	243	241
Piazza Santa Croce	30	31	32	47	48	49
Piazza Signoria	0	0	0	36	36	36
Via Romana	34	34	31	203	203	200
Piazza San Lorenzo	4	4	1	60	60	57
Piazza Ognissanti	4	7	7	125	128	128
Via Sguazza	6	6	6	50	50	50
Santa Maria del Fiore	2	4	4	18	20	20
Via di Camporeggi	18	20	19	48	50	49
<i>Total</i>	<i>347</i>	<i>406</i>	<i>364</i>	<i>1880</i>	<i>1939</i>	<i>1897</i>

Table III: Statistical analysis relative to pollen data, sampling site and the number of Cupressaceae trees (* p-value < 0.05).

<i>Buffers 250 m</i>	<i>DF</i>	<i>SS</i>	<i>F</i>	<i>p-value</i>	<i>Adj Rsq</i>
Sampling site	1	0.79	4.83	0.02*	0.03
Cupressaceae/Taxaceae trees	1	0.94	6.06	0.01	0.05
<i>Buffers 500 m</i>					
Sampling site	1	0.78	4.79	0.03*	0.03
Cupressaceae/Taxaceae trees	1	0.34	2.10	0.15	0.02



6. Contribution of land cover and wind to the airborne pollen recorded in a South-European urban area

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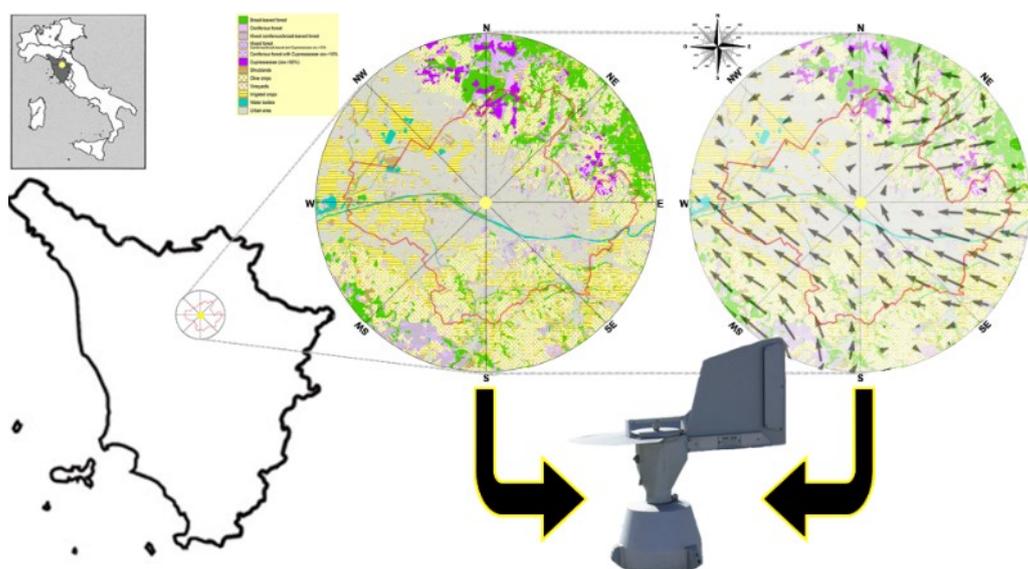
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Abstract

Airborne pollen assemblage depends on the land cover of the area surrounding the volumetric trap and the flow of air masses. In urban contexts, the amount of airborne pollen is the result of the contribution of both local green areas and extra-urban vegetation, in addition to wind direction and intensity. The present study focused on the combined effects of land cover and wind on the airborne pollen recorded in the city of Florence over a five-year period, in order to identify the area with the greatest pollen contribution. The study revealed that Cupressaceae, *Olea*, and *Quercus* pollen display a positive correlation among their pollen values and the geographical distribution of their sources. The overall influence of maximum wind intensity is greater than the number of calm wind days. The latter is appreciable only for those pollen grains (Cupressaceae and *Olea*) whose trees are located in the proximity of the urban area, in the southern part of the city. The results showed that the land-cover configuration strongly influences the airborne pollen captured by volumetric traps; despite the prevalent wind directions, the vegetation of the reliefs surrounding the city can be considered as the greatest area of pollen contribution.

Keywords: Pollen forecasting; Land-cover; wind-data; GIS; urban environment; Southern Europe



Graphical abstract

Cover image: View of the Florence plain from Monte Morello.

1. Introduction

Human activities strongly shape the landscape: this is particularly evident in southern Europe, where only 4.7% of the primary plant communities have been preserved unaltered (Geri et al. 2010). Every change in land cover has a decisive impact on the local airborne pollen composition. In addition to the plants growing in the area, several other factors affect pollen records, including topography, latitude and weather variables (Rojo et al. 2015).

The forecast of airborne particles must absolutely take into account the weather variables, since several meteorological factors such as wind, rainfall, air temperature and humidity strongly influence pollen diffusion (Damialis et al. 2005). Airflow, in particular, has been recognised as one of the major factors affecting pollen circulation in the atmosphere (Palacios et al. 2000), given that wind direction and speed determine the pollen dispersion distance which may be noticeable. In some cases, long-distance transport can cause pre-seasonal peak values, as often recorded in Denmark, where large quantities of birch pollen arrived from Poland or Germany before local blooming (Skjøth et al. 2007). Wind may also cause the diffusion of high pollen amounts coming from plants located in the neighbourhood (Palacios et al. 2000): this is particularly frequent in urban areas, where buildings can modify the local airflow and cause turbulence or convection currents (Damialis et al. 2005).

In recent years, Geographic Information System (GIS) software has become an important analytical tool in aerobiological studies, offering the possibility to examine many factors associated with airborne pollen (Oteros et al. 2015; Rojo et al. 2015; García-Mozo et al. 2016; Maya-Manzano et al. 2017). The availability of detailed maps of allergenic plants provides a necessary device for a correct health impact assessment, allowing sensitised people to reduce their exposure and limit risks (McInnes et al. 2017). The knowledge of the relationships between the distribution of plants and their airborne pollen may be of fundamental importance for evaluating potential risk in clinical surveys and in daily recommendations for sensitised subjects (Fernández-Rodríguez et al. 2014).

In this paper, we investigated the relationships between airborne pollen, wind and land cover in the surroundings of Florence (NE Tuscany, Italy), in order to assess the areas of greatest pollen

contribution in the city. We focused on the pollen of Cupressaceae, Pinaceae, *Castanea*, *Olea*, and *Quercus*: these plants are widespread in urban and extra-urban areas and their pollen grains are the most frequently observed, both in the aerobiological spectra of central Italy and in other Mediterranean countries (Mincigrucci et al. 1985; Frenguelli et al. 2002; Puljak et al. 2016). More specifically, the aims of this study were to examine the pollen contribution of these plants and the relationship between their local distribution and wind direction and intensity.

2. Material and methods

2.1 Study site

Florence (43°47'14.64" N, 11°14'59,64" E, 50 m above sea level (a.s.l.)) (Figure 1) lies on the central part of the Arno-river basin, an extensive plain of about two thousand hectares (ha) surrounded by hills of medium-low height (400-500 m a.s.l.). The Arno river crosses the city along a longitudinal line.

The climate of Florence is characterised by dry and hot summers, wet autumns and springs, and cold winters (Petralli et al. 2011). The annual average temperature is 15.5° C. The coldest month is January (average temperature 6.8° C); the hottest is July (average temperature 25.3° C). The average annual rainfall is around 830 mm: in recent years, the wettest month has always been November (average of 105.4 mm rain) (Borchi & Macii 2015).

The vegetation of the urban area is dominated by ornamental trees and shrubs growing along the roads or by public and private green spaces. The most used tree for ornamental purposes is *Tilia* (15% of the total road trees), followed by Cupressaceae (14%) and *Quercus* (13%) (Municipality of Florence, Territorial Information System 2016). In the area surrounding the city, the land-cover is mainly represented by olive groves, irrigated crops and vineyards. Natural vegetation can be found as *Quercus pubescens*-dominated woodlands, shrublands and grasslands.

2.2 Selected plants and pollen grains

Cupressaceae. Cupressaceae are widely used for an ornamental purpose in the urban green areas of the municipality of Florence and for reforestation in the hills surrounding the city (*i.e.* Monte Morello, Monte Cagnani-Val Marina, Monte Ceceri) (Vv.Aa. 2009). Cupressaceae pollen grains usually dominate the pollen spectra for a large part of the year in many Mediterranean areas, due both to their mass flowering and the prolonged pollen season of different species (Diaz de la Guardia et al. 2006). Several species of this family, mainly *Cupressus sempervirens* L., have been identified as one of the predominant causes of allergic syndrome during the winter (Nardi et al. 1996).

Pinaceae. *Abies*, *Picea*, and *Pinus* are the most common genera of Pinaceae in the studied area. *Pinus* species are largely used for reforestation and for ornamental purposes, especially in Tuscany, where the most used species for resettlement are *Pinus pinea* L., *Pinus nigra* J.F Arnold and *Pinus pinaster* Aiton. The pollen of these anemophilous plants is commonly recorded by pollen traps, where they are often underrepresented (Räsänen et al. 2004).

Castanea. *Castanea sativa* Mill. is common in the woods of Tuscany, especially on sandy soils (Arrigoni and Viciani 2001). This plant is characterised by an elevated pollen production and by mainly anemophilous pollination (Mandrioli, et al. 1977). Due to these characteristics, combined with their small size and low fall speed (Mandrioli et al. 1977), these pollen grains have a high dispersion capacity and are widely studied for medium-long range pollen transport studies (Jato et al. 2001).

Olea. The countries around the Mediterranean basin host the largest amount of olive groves of the world (Loumou & Giourga 2003). Olive trees are largely cultivated in Tuscany, with 35% in the province of Florence (Maselli et al. 2012). *Olea* pollen grains are among the most abundant airborne particles in southern Europe (Garcia Mozo et al. 2016): their shape, size and large amount make them particularly suitable for studying atmospheric dispersion models (Fernández-Rodríguez et al. 2014)

Quercus. *Quercus* species (*Quercus cerris* L. *Quercus pubescens* Willd. and *Quercus ilex* L.), are the dominant trees of the broad-leaved forests that cover almost half the province of Florence (Vv.Aa. 2005). Oaks are anemophilous plants that produce a high quantity of pollen (Grewling et al. 2014):

their grains may cause respiratory allergies due to a cross-reactivity reaction with other pollen grains such as *Alnus*, *Betula*, *Castanea*, *Olea* and *Poaceae* (Recio et al. 2018).

2.3 Airborne pollen data

Airborne pollen was continuously monitored by means of a 7-day Hirst-type volumetric spore trap (Hirst 1952; model Lanzoni VPPS-2000) placed on the roof (about 20 m above ground level). Pollen data were furnished by the Regional Agency for Environmental Protection of Tuscany (ARPAT), unit of Florence, located in the western part of the city (Figure 2). The data concern the time intervals from 2011 to 2015 and were freely downloaded from the website of ARPAT (<http://www.arpato.toscana.it>).

Pollen data are expressed as daily pollen concentration, *i.e.* the number of airborne pollen grains per unit volume of air (n. pollen grains/m³). The Main Pollen Season (MPS) of the plants, *i.e.* the time interval of significant concentration of its pollen in the atmosphere (Galan et al. 2017), was calculated following the method suggested by Andersen (1991). MPS begins when 2.5% of its total annual pollen amount is recorded and ends when 97.5% of the total annual pollen is reached. For each plant studied the start-end days, the length, the total pollen sum, *i.e.* the total annual pollen grains, and the peak value of the MPS were reported. All the data were calculated using the ‘AeRobiology’ package (Rojo et al. 2018) with R-Studio software (R Core Team 2018).

2.4 Land cover and plant distribution

In order to localise the nearest pollen source plants in the surroundings of Florence, the land-cover has been analysed in an area of more than 300 km². A buffer of 10 km in radius surrounding the pollen monitoring station was drawn using the ESRI ArcMap 10.6. For an immediate correlation with meteorological variables and the pollen data, the buffer was divided into eight parts (octants) corresponding to the predominant wind directions, defined as the origin from which the wind blows: north-northeast (N-NE), northeast-east (NE-E), east-southeast (E-SE), southeast-south (SE- S),

south-southwest (S-SW), southwest-west (SW-W), west-northwest (W-NW), northwest-north (NW-N) (Fig. 2). The land cover was classified combining orthophoto interpretation and the use of WMS layers provided by the Territorial and Environmental Information System of Tuscany. The orthophotos used for this study refers to the year 2013, the central period of the pollen sampling period. Different layers were separated depending on the land cover of the octants. The total area of the identified land-cover categories and the relative quantities in each octant were calculated using the GIS software: the measures are expressed as km² and percentages, relative of each octant and as the total buffer area.

2.5 Wind data

The daily wind data (mean direction, maximum and mean intensity) were supplied by the thermo-pluviometrical station of the University of Florence (43°47'56.97" N, 11°15'04.46" E, 84 m a.s.l.), located in the north-eastern area of the city, about 2 km from the pollen monitoring station (Fig. 2). The days with mean values of wind intensity lower than 1 m/s were considered as “calm days”. All the data were freely downloaded from the internet site of Regional Hydrological Service (SIR) of Tuscany (<http://www.sir.toscana.it>).

2.6 Statistical analysis

The correlation between pollen data and meteorological variables was estimated to understand the influence of wind direction and intensity during the MPS. For this purpose, two different types of generalised linear models (GLM) were chosen. The first model (Model-1) correlates the airborne pollen records of each selected plant during its MPS with the daily wind mean directions and the wind maximum intensities registered during the same period. The second model (Model-2) correlates the same airborne pollen records with the daily wind mean directions and the frequency of calm wind days recorded during the MPS of the selected plant. These models analyzed, as a whole, the MPSs of

the entire time interval (2011-2015). The pollen values were log-transformed in order to normalize the data. Statistical correlations were made using R-Studio software (R Core Team 2018).

3. Results

3.1 Airborne pollen

The pollen record of the selected plants for the time interval 2011 – 2015 is shown in Table I. Figure 3 shows the annual trend recorded during the entire analyzed period.

Cupressaceae MPS starts at the beginning-mid February and ends in mid-April. The highest total pollen sum and maximum daily peak value was reached in 2011 (respectively 88493 grains/m³ and 8944 grains/m³), and the lowest in 2013 (respectively 26655 grains/m³ and 2256 grains/m³).

Pinaceae MPS starts at the beginning of April and ends in the middle of July, with some peaks in October and November. The highest total pollen sum was recorded in 2015 (3770 grains/m³), and the lowest in 2013 (1283 grains/m³). The highest daily peak value was recorded in 2015 (326 grains/m³), and the lowest in 2011 (87 grains/m³).

Castanea MPS starts at the beginning of June and ends in the middle of August. The highest total pollen sum and daily peak value were reached in 2011 (respectively, 1662 grains/m³ and 162 grains/m³), and the lowest in 2014 (respectively, 404 grains/m³ and 34 grains/m³).

Olea MPS starts in the mid-late May and ends in mid-late June. The highest total pollen sum and daily peak value were reached in 2013 (respectively 4326 grains/m³ and 761 grains/m³); with the lowest in 2014 (respectively 569 grains/m³ and 42 grains/m³).

Quercus MPS starts in early-mid April and ends at the beginning of June. The highest total pollen sum and daily peak value were reached in 2015 (respectively, 13125 grains/m³ and 1101 grains/m³), with the lowest in 2013 (respectively, 7766 grains/m³ and 457 grains/m³).

3.2 Land cover and plant distribution

The orthophoto interpretation allowed us to identify twelve main land cover categories (Table II), and the extensions of these categories are reported in Table III. Figure 4 shows the land cover map of the buffer area.

The largest land cover type was “Artificial surfaces”, *i.e.* the urban area, with more than 100 km² of extension (35% of the total area buffer); the highest value was shown by the octant W-NW (20.3 km²), and the lowest by the octant N-NE (7 km²).

The second largest land cover category was “Agricultural areas”, including areas with olive crops, and reached an overall extension of 64.6 km² (21% of the total area): the highest values were reached in the NE-E and SE-S octants (more than 12 km²), with the lowest in the W-NW octant (0.02 km²).

Among the “Forests and semi-natural areas”, broad-leaved forests covered almost 34 km² of the total buffer area (11%) and the highest surface extension was reached in the octant N-NE (10.5 km²).

Mixed coniferous/broad-leaved forest covered more than 14 km² of the total surface (4.5%) and the highest extension was reached in the octant S-SW (5.15 km²). Cupressaceae thickets with a coverage > 50% and coniferous forest showed similar values (6 km² of extension, 2% of the total area). These two types were also present as mixed formations in other land cover categories, such as coniferous reforestations with Cupressaceae coverage < 10% or mixed with other coniferous, broad-leaved trees and with Cupressaceae coverage < 10% (percentages close to 0.5-1%).

3.3 Wind direction and intensity

The meteorological data recorded during the MPS of the selected pollen taxa during 2011 – 2015 are reported in Table IV.

A prevalence of southeast or north-east mean directions was alternately observed during Cupressaceae MPSs. The most frequent mean wind directions were east and north-east during *Castanea*, Pinaceae and *Quercus* MPSs. During *Olea* MPSs the wind mean direction was east, except in 2011 when the mean direction was south-east.

The greatest number of calm wind days was reached during the Pinaceae MPS in 2014 (54 days), with the lowest during *Castanea* MPSs (0 days in 2011, 2012 and 2013) and *Olea* MPSs (0 days in 2011 and 2012).

Wind maximum intensity was reached during Cupressaceae MPS in 2015 (26.2 m/s); high values were also recorded during the MPS of the other plants. The lowest wind intensity was observed in 2012, during *Castanea* MPS (6.1 m/s).

3.4 Statistical results

The results from the statistical models and pollen data recorded during MPS in the period 2011-2015 are reported in Table V. The table lists only the correlations with statistical significance. The highest correlation was shown with northern and southern wind directions. The significance increased when considering the variable wind maximum intensity (Model-1) rather than calm wind days' (Model-2). The greatest number of significant correlations was shown by Pinaceae pollen, the lowest by Cupressaceae.

4. Discussion

4.1 Airborne pollen trends

The airborne pollen records allowed us to identify the period of maximum pollen diffusion of the selected plants in the urban area during the time interval 2011 – 2015 (Figure 3).

Cupressaceae MPS shows an average of more than fifty days (from the middle of February) and is roughly in agreement with those recorded in other Mediterranean regions (Elvira-Rendueles et al. 2019). The high pollen production is highlighted by the mean value of the yearly total pollen sum (46000 grains), the highest among those considered in this paper (Fig. 3). This massive pollen diffusion makes Cupressaceae pollen the most frequent in aerobiological spectra during the year (Diaz de la Guardia et al. 2006), and causes an increasing allergen sensitisation, as recorded in many Italian regions (Sposato et al. 2014). In recent years, Cupressaceae pollen concentration showed a substantial

increase in Spain, probably due to a considerable increase in temperature, with an important advance in the start of the MPS (Subiza et al. 2018). This trend was not recorded in Florence, where Cupressaceae MPS displayed a progressive decrease and a delay of its end rather than an advanced beginning. The dissimilarities could be explained by the increase in rainfall during the months prior to anthesis (Galan et al. 1998).

Pinaceae has the longest MPS among those analysed, with a mean length of 160 days. The length of the pollination period can be due to the sum of the blooming periods of the different genera of this family. Pinaceae pollen concentrations often show two distinct peaks during the year: the former at the beginning of spring, due to the blooming of *Pinus* and *Abies*, and the latter at the beginning of autumn, due to the blooming of *Cedrus* (De Linares et al. 2017; Elvira-Rendueles et al. 2019). Pinaceae pollen production depends on climatic conditions; Jato et al. 2000 suggested that rainfalls increase the length of the Pinaceae pollination period. This hypothesis agrees with our data: the longest MPSs (up to the second half of October in 2013 and 2014) correspond to a high annual daily average rainfall, with the exception of 2011 (data from the Ximenian Observatory, <http://www.ximeniano.it/>). Unlike the end of MPS, the beginning of MPS is quite stable over time and similar to those recorded in other Mediterranean cities (Spain: De Linares et al. 2017; Greece: Gioulekas et al. 2004; Italy: Romano et al. 1988).

The length of *Castanea* MPS (ca. 46 days, from the beginning of June) is approximately the same as that recorded in Trieste, NE Italy (Rizzi-Longo et al. 2005), but differs from that recorded by Rodríguez de la Cruz et al. (2008) and Astray et al. (2016) in Spain. Moreover, *Castanea* pollen grains can often be found in the atmosphere, even after the flowering period, leading to peaks out of season (Rodríguez de la Cruz et al. 2008).

Olea MPS approximately starts at the end of May and finishes in the first half of June (mean of 28 days). The same period is reported for Perugia, another town in central Italy (Bonofiglio et al. 2009). The high annual pollen amount (about 2500 grains/m³) recorded here reflects the widespread presence of olive groves but is much lower than that recorded in south-western Spain, where intensive olive-

growing practices are responsible for the highest *Olea* pollen values in the Mediterranean region (Skjøth et al. 2013). It is important to underline the very low *Olea* pollen concentration (total pollen sum 569 grains/m³) recorded during 2014, possibly due to climatic anomalies during Autumn 2013 – Spring 2014 (Zanoni et al. 2016).

Quercus presents a mostly steady MPS (ca. 60 days, from the first half of April), as also observed in Malaga, Spain, during the same period (2011 – 2015; Recio et al. 2018). *Quercus* pollen shows the second highest annual amount (average of 10000 grains/m³) after Cupressaceae; this value reflects the large diffusion of these plants in the area surrounding Florence, unlike other countries where they are less widespread (Corden & Millington 1999). It is to note that *Quercus* total pollen levels vary over the years. This trend has also been recorded in other European regions (Spieksma et al. 2003) and is probably due to the airborne period of these pollen grains which primarily depends on the weather conditions (Jäger et al. 1991).

4.2 Wind data and pollen sources

The statistical analysis of the daily pollen concentration and wind direction and intensity, combined with the spatial analysis of the land cover (Figure 4), allowed us to identify the area of greatest pollen contribution to the urban area.

The daily pollen concentrations of Pinaceae, *Castanea*, *Olea* and *Quercus* are strongly related with the winds blowing from the north and south. Therefore, the plants growing on the hills of the north and south sides provide a higher pollen amount than those growing on the hills of the eastern octants, even if east represented the most frequent wind direction during the MPS of these plants. Northern and southern reliefs facing the city provided the greatest pollen sources.

The mixed effect of maximum wind intensity and north westerly wind direction show a strong positive correlation with the recorded pollen values of Cupressaceae (Model-1, Table V). Therefore, the effect of maximum wind intensity seems to have a strong positive influence on Cupressaceae pollen recorded in the urban area, as already observed by Gioulekas et al. (2004). The cross-check

with the buffer area hit by the north westerly wind direction shows a noticeable extension of Cupressaceae woods, second only to the north eastern area of the buffer. On the contrary, Cupressaceae pollen values show a statistical correlation with the frequency of calm wind days combined with the south easterly wind direction (Model-2, Table V). The presence of numerous cypress trees near to the volumetric spore trap (Figure 4) may justify a greater Cupressaceae pollen contribution from the Southern hills when the wind blows at a low intensity. The contribution of these plants has already been demonstrated by a recent study conducted in the city (Ciani et al. 2019). Pollen concentrations are usually positively related to calm wind periods, particularly when its source is located in the immediate vicinity of the sampling sites (Palacios et al. 2007). Therefore, both maximum and low wind intensities seem to have a significant influence on Cupressaceae pollen, despite previous studies that showed a weak correlation (Damialis et al. 2005; Rojo et al. 2015).

The maximum wind intensity seems to have an even more significant effect when considering Pinaceae MPSs. Indeed, a considerable positive correlation is shown with the mixed effect of combining the maximum wind intensity variable with wind directions from the North, South and East (Model-1, Table V). The land cover map (Figure 4) shows a similar area covered by coniferous formations (both pure and mixed) in northern octants and southern octants; the surface is, instead, smaller in the eastern part of the buffer. Therefore, the plants growing in the areas to the North and South of the city seem to contribute significantly to the Pinaceae pollen concentration recorded in the urban area. It is to note that wind direction generally affects the diffusion of Pinaceae pollen (Table V), highlighting the tendency of the saccate grains to be easily airborne (Schwendemann et al. 2007). A scarce and negative correlation is instead observed between Pinaceae pollen values and the frequency of calm wind days (Model-2, Table V), as already observed by Jato et al. (2000). This evidence suggests a greater pollen contribution when winds blow at a high intensity and that these pollen grains are more often subjected to long-distance transport episodes (Szczepanek et al. 2017). *Castanea* pollen concentration shows a significant positive correlation with the northerly wind direction (Model-1, Table V). However, the correlation becomes considerably negative when wind

direction is taken into account together with maximum wind intensity. Combining these results with the land cover, the Northern area of the buffer shows the largest extension of broad-leaved forests. A negative correlation between pollen values and the main source area of chestnut woods has already been observed (Jato et al. 2001) and the authors suggested pollen transport over a greater distance than in the nearby area. The medium-long range transport of *Castanea* pollen was detected in Switzerland by Frei (1997) and, in this case, the nearest chestnut pollen sources were far more than a hundred kilometres away.

Olea shows a strong positive correlation when the combined effect of the north westerly wind direction and maximum wind intensity variables are taken into account (Model-1, Table V). Olive crops in the North Western octants are very scarce, so the pollen contribution from this area seems not to be considerable, and olive pollen grains might be transported from a longer distance than that considered for this study. A good correlation is also observed combining the north easterly wind direction with calm wind days (Model-2, Table V). The north eastern area of the buffer shows a widespread presence of olive trees (about 7% of the total area), suggesting a great pollen contribution from this part when the wind blows at low intensity. *Olea* pollen shows a predominance of medium-long distance transport (Maya-Manzano et al. 2017), and Fornaciari et al. (2000) suggested that olive trees more than 50 km away from the sampling point may contribute to pollen values during favourable meteorological conditions. Nevertheless, it is difficult to assess the provenance of olive pollen; in fact, these grains have local diffusion in stable conditions and long-medium distance transport in the presence of continuous and intense air-flows (Hernández-Ceballos et al. 2011). The continuous change in *Olea* flowering period causes a problem in forecasting olive pollen behaviour, as exemplified by the years strongly influenced by climatic changes. In particular, an increase in temperature led to a progressive lengthening of the pollen season (García-Mozo et al. 2014).

Quercus pollen concentrations show an overall statistical correlation with northerly wind directions combined with maximum wind intensity (Model-1, Table V). The Northern area of the buffer shows a widespread diffusion of broad-leaved forests, in particular in North Eastern octants where these

formations (both pure and mixed) reached the highest extension. This evidence, combined with the positive statistical correlation, confirms a significant pollen contribution from the area where these plants are widespread (Moreno-Grau et al. 2000). The same positive correlation is observed for a southerly mean wind direction and, in this case, the widespread diffusion of broad-leaved formations in Southern octants suggests a fair contribution from these areas. A negative correlation is instead observed when the mixed effect of the northerly mean wind direction and wind calm days are jointly considered (Model-2, Table V). The opposite influence of maximum wind intensity and calm wind days was already observed by Recio et al. (2018). Although in previous studies (Corden & Millington 1999; Maya-Manzano et al. 2017), wind direction and intensity were not found to be significant for airborne *Quercus* pollen, in this case its influence must be taken into account. Combining the results of both statistical models and land cover analysis, the greatest *Quercus* pollen contribution comes from the Northern reliefs that surround the city when the wind blows at a significant intensity.

5. Conclusions

The pollen monitoring carried out during the years of study made it possible to evaluate the periods of maximum pollen diffusion of five *taxa* which are widespread in the urban area and surroundings: Cupressaceae, Pinaceae, *Castanea*, *Olea*, and *Quercus*. The main pollen seasons are generally in line with those recorded in other European countries, apart from slight differences due to the different latitudes and climatic conditions.

This study shows that statistical analysis between pollen values and wind data, combined with land-cover analysis, can provide a valuable tool for understanding the dynamics of pollen transport, despite the difficulties in assessing the influence of the flow of air masses on airborne pollen trajectories. Moreover, this joint analysis allows us to evaluate the actual pollen contribution from the area surrounding the sampling point.

The overall picture that comes from the results shows us that probably the greatest pollen contribution in Florence is provided by the vegetation of the Northern reliefs. This is particularly evident for

Cupressaceae, *Olea*, and *Quercus* pollen grains when the wind blows at a high intensity. Moreover, the positive correlation with meteorological variables also corresponds to the buffer areas with the greatest coverage of their pollen sources. In other cases, the pollen transport associated with high wind intensity is not easily explainable, as shown with different results for Pinaceae and *Castanea* pollen grains: Pinaceae pollen diffusion is mainly due to the high capacity of these pollen grains to float in the air; *Castanea* pollen provenance is difficult to detect, because of the considerable amount of grains transported over a long-distance. Therefore, the local contribution from nearby pollen sources is more appreciable in periods of calm wind only for some plants, as has been observed for Cupressaceae and *Olea* pollen grains.

Figure 1: Geographical location of Florence.

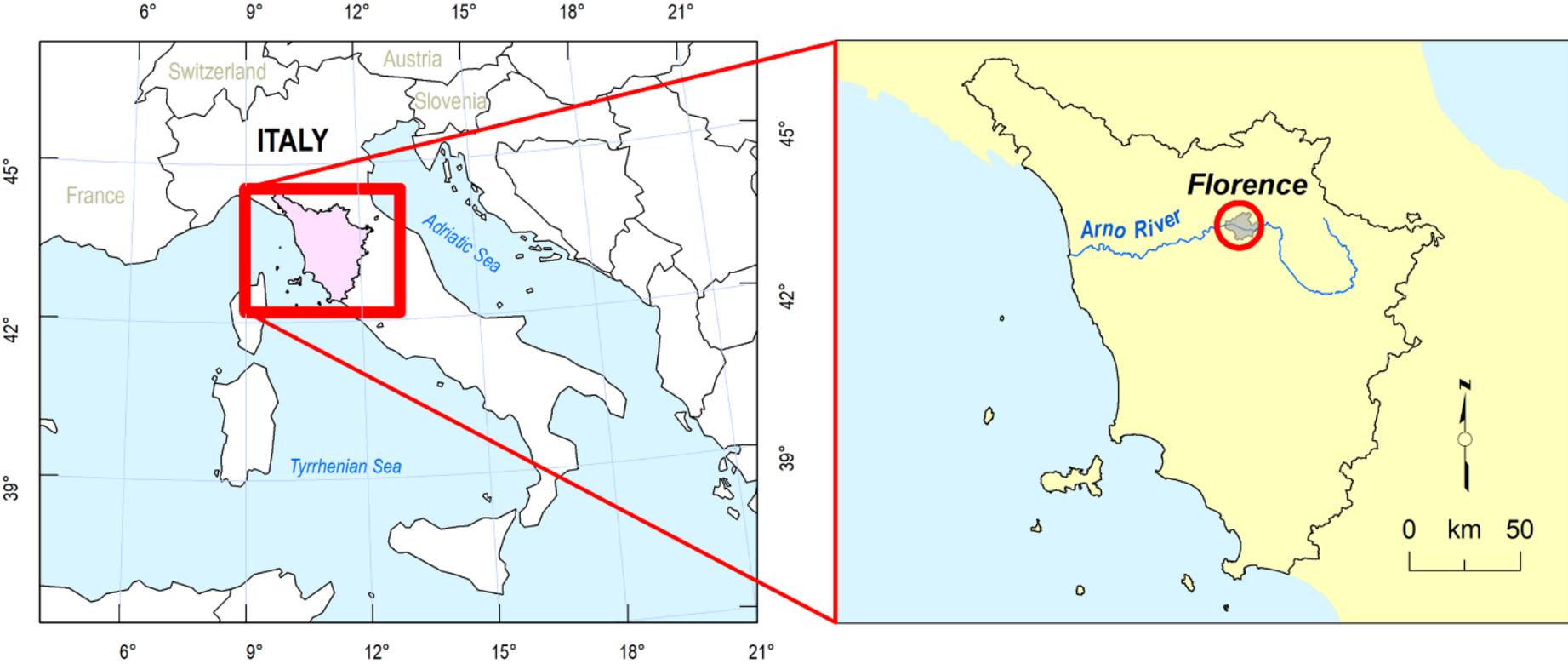


Figure 2: The studied area surrounding the pollen sampling station.

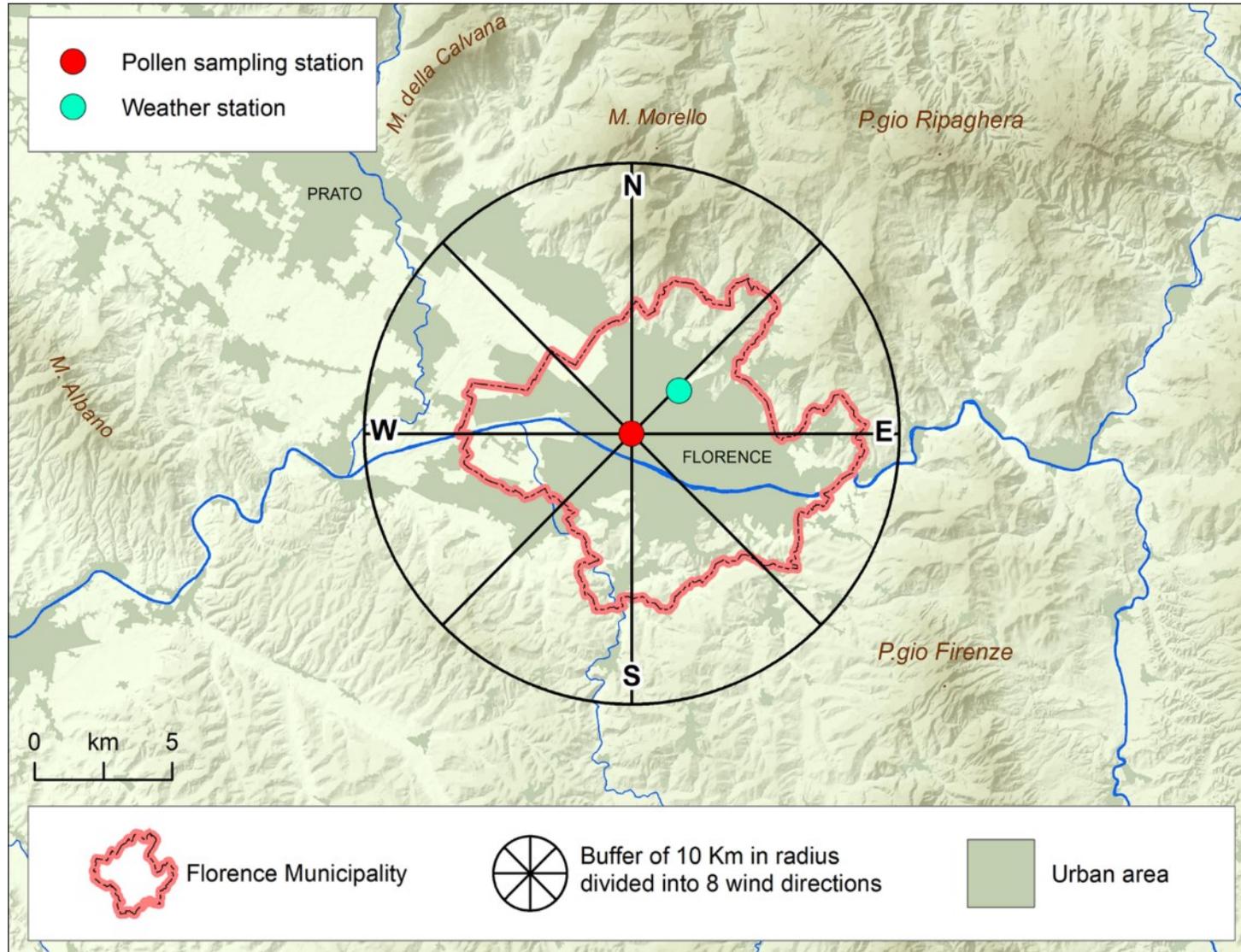


Figure 3: Average of the daily pollen concentration recorded for each taxon during the study period (2011 – 2015). The bar plot at the bottom right shows the average of the yearly total pollen sum.

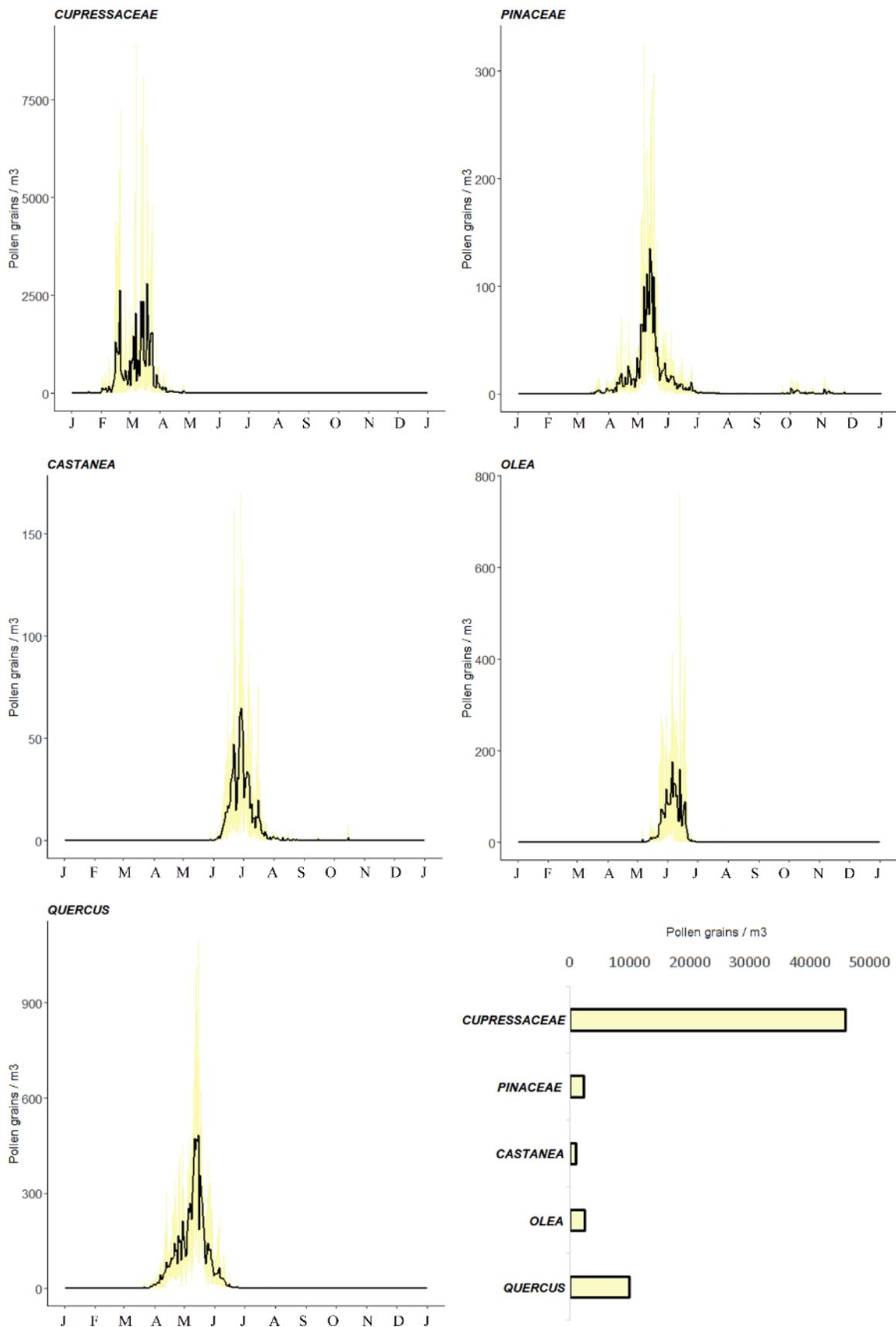


Figure 4: Land cover map of the studied area.

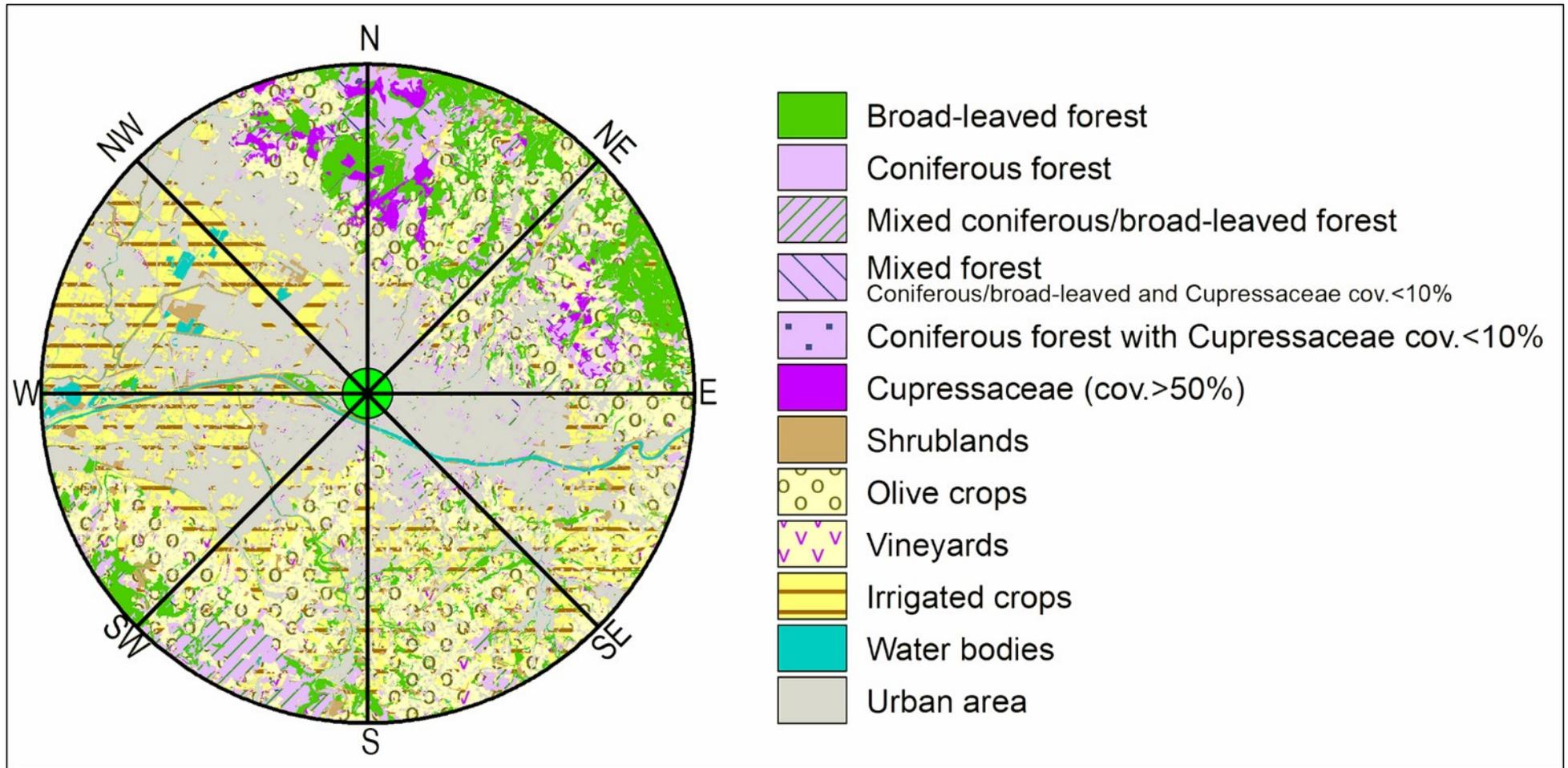


Table I: Airborne pollen data of the time interval 2011-2015. Total pollen sums and peaks values are expressed as pollen grains/m³.

	2011	2012	2013	2014	2015	mean
Cupressaceae						
<i>Main pollen season–(MPS)</i>	11/02 – 04/04	23/02 – 03/04	16/02 – 26/04	06/02 – 29/03	12/02 – 17/04	
<i>MPS length (days)</i>	53	41	70	52	65	56
<i>Total pollen sum</i>	88493	32548	26655	48813	33420	46000
<i>Peak value – date</i>	8944 – 07/03	3834 – 18/03	2256 – 23/03	7280 – 19/02	3042 – 20/03	
Pinaceae						
<i>Main pollen season–(MPS)</i>	05/04 – 22/10	03/04 – 14/07	15/04 – 17/10	22/03 – 18/10	11/04 – 10/07	
<i>MPS length (days)</i>	201	103	186	211	97	160
<i>Total pollen sum</i>	2143	2432	1283	2087	3770	2343
<i>Peak value – date</i>	87 – 10/05	284 – 13/05	155 – 11/05	169 – 04/05	326 – 07/05	
Castanea						
<i>Main pollen season–(MPS)</i>	09/06 – 03/08	15/06 – 20/07	18/06 – 10/08	06/06 – 18/07	11/06 – 22/07	
<i>MPS length (days)</i>	56	36	54	43	42	46
<i>Total pollen sum</i>	1662	980	958	404	1180	1037
<i>Peak value – date</i>	162 – 22/06	170 – 27/06	92 – 06/06	34 – 04/07	67 – 25/06	
Olea						
<i>Main pollen season–(MPS)</i>	21/05 – 06/06	24/05 – 20/06	01/06 – 25/06	14/05 – 17/06	13/05 – 14/06	
<i>MPS length (days)</i>	17	28	25	35	33	28
<i>Total pollen sum</i>	2490	2775	4326	569	2438	2519
<i>Peak value – date</i>	280 – 25/05	414 – 04/06	761 – 13/06	42 – 03/06	295 – 06/06	
Quercus						
<i>Main pollen season–(MPS)</i>	09/04 – 27/05	05/04 – 10/06	16/04 – 05/06	04/04 – 07/06	15/04 – 04/06	
<i>MPS length (days)</i>	49	67	51	65	51	57
<i>Total pollen sum</i>	11609	8879	7766	8569	13125	9990
<i>Peak value – date</i>	714 – 11/05	458 – 28/04	457 – 14/05	768 – 11/05	1101 – 15/05	

Table II: Land cover categories detected in the 10 km radius buffer surrounding the volumetric sampler and the related pollen sources. cov. = coverage.

Land cover type	Land cover category	Description	Pollen source
Forests and semi-natural areas	Broad-leaved forest	Vegetation formation composed principally of trees where broad-leaved species (mostly) predominate. Riparian vegetation has also been included	<i>Quercus</i> sp., <i>Castanea sativa</i>
	Coniferous forest	Vegetation formation composed principally of trees where coniferous species predominate	Pinaceae
	Mixed coniferous/broad-leaved forest	Vegetation formation composed principally of trees where neither broad-leaved nor coniferous species predominate	<i>Quercus</i> sp., <i>Castanea sativa</i> , Pinaceae
	Mixed forest (coniferous, broad-leaved and Cupressaceae cov. < 10%)	Vegetation formation composed principally of trees where neither broad-leaved nor coniferous species predominate and Cupressaceae cover less than 10%	<i>Quercus</i> sp., Pinaceae, <i>Cupressus</i> sp.
	Coniferous forest with Cupressaceae cov. < 10%	Vegetation formation composed principally of coniferous species with the presence of Cupressaceae cover less than 10%	Pinaceae, <i>Cupressus</i> sp.
	Cupressaceae cov. > 50%	Vegetation formation composed principally of trees where Cupressaceae cover more than 50%	<i>Cupressus</i> sp.
Agricultural areas	Shrublands	Mediterranean and sub-Mediterranean evergreen sclerophyllous bushes and shrubs	
	Olive crops	Area mainly used as olive groves	<i>Olea europea</i>
	Vineyards	Area mainly used for vine cultivation	
	Irrigated crops	Cultivated areas under agricultural use for arable crops that are permanently or periodically irrigated	
Water bodies	Inland waters	Natural or artificial water courses/bodies	
Artificial surfaces	Urban area	Area mainly occupied by dwellings, buildings, roads, etc.	

Table III: Land cover results of the 10 km radius buffer.

Land cover category	N - NE		NE - E		E - SE		SE - S		S - SW		SW - W		W - NW		NW - N		Total	
	<i>km²</i>	%																
Broad-leaved forest	10.46	3.3	9.15	2.9	1.02	0.3	3.10	1.0	3.09	1.0	2.64	0.8	0.54	0.2	3.80	1.2	33.80	10.8
Coniferous forest	2.24	0.7	0.91	0.3	0.10	0.0	0.53	0.2	1.33	0.4	0.10	0.0	0.08	0.0	0.36	0.1	5.65	1.8
Mixed coniferous/broad-leaved forest	2.25	0.7	1.93	0.6	1.24	0.4	1.33	0.4	5.15	1.6	1.08	0.3	0.33	0.1	1.00	0.3	14.31	4.5
Mixed forest (coniferous, broad-leaved and Cupressaceae cov. < 10%)	0.84	0.3	0.78	0.2	0.43	0.1	0.15	0.0	0.26	0.1	0.19	0.1	0.03	0.0	0.98	0.3	3.66	1.2
Coniferous forest with Cupressaceae cov. < 10%	0.66	0.2	0.40	0.1	0.01	0.0	0.08	0.0	0.09	0.0	0.01	0.0	0.01	0.0	0.21	0.1	1.48	0.5

Table III (continued)

Land cover category	N - NE		NE - E		E - SE		SE - S		S - SW		SW - W		W - NW		NW - N		Total	
Cupressaceae cov. > 50%	2.65	0.8	1.34	0.4	0.12	0.0	0.14	0.0	0.09	0.0	0.06	0.0	0.07	0.0	2.13	0.7	6.59	2
Shrublands	0.67	0.2	0.43	0.1	0.77	0.2	0.97	0.3	1.07	0.3	1.67	0.5	2.69	0.9	0.61	0.2	8.89	2.8
Olive crops	9.54	3.0	12.24	3.9	8.57	2.7	12.32	3.9	11.16	3.6	4.84	1.5	0.02	0.0	5.95	1.9	64.64	20.6
Vineyards	0.23	0.1	0.33	0.1	0.58	0.2	1.96	0.6	1.88	0.6	1.47	0.5	0.08	0.0	0.12	0.0	6.65	2.1
Irrigated crops	2.64	0.8	2.13	0.7	6.26	2.0	8.34	2.7	5.93	1.9	10.10	3.2	13.56	4.3	5.28	1.7	54.23	17.3
Inland water	0.04	0.0	0.07	0.0	0.84	0.3	0.32	0.1	0.26	0.1	0.99	0.3	1.59	0.5	0.30	0.1	4.41	1.4
Urban area	7.04	2.2	9.55	3.0	19.32	6.2	10.03	3.2	8.94	2.8	16.14	5.1	20.29	6.5	18.53	5.9	109.85	35

Table IV: Meteorological data concerning mean daily direction, frequency of calm days and maximum daily intensity of wind during 2011-2015.

	2011	2012	2013	2014	2015	2011-2015 (mean)
Cupressaceae MPS						
<i>Mean direction</i>	NE	SE	NE	SE	NE	NE - SE
<i>Calm days</i>	7	7	6	6	14	8
<i>Max intensity</i>	11.4	9.4	11.6	8.5	26.2	13.4
Pinaceae MPS						
<i>Mean direction</i>	E	E	E	E	E	E
<i>Calm days</i>	8	6	12	54	22	> 20
<i>Max intensity</i>	9.2	10.7	8.8	16.4	20	13
Castanea MPS						
<i>Mean direction</i>	E	E	E	E	E	E
<i>Calm days</i>	0	0	0	4	5	< 2
<i>Max intensity</i>	7.1	6.1	8.8	14.1	20	11.2
Olea MPS						
<i>Mean direction</i>	SE	E	E	E	E	E
<i>Calm days</i>	0	0	1	5	4	2
<i>Max intensity</i>	7.9	4.8	7.3	14.1	14	9.6
Quercus MPS						
<i>Mean direction</i>	NE	E	E	E	E	E
<i>Calm days</i>	2	5	3	18	16	~ 9
<i>Max intensity</i>	9.2	10.7	7.7	16.4	14.3	11.7

Table V: Statistical results between pollen data and wind. Model-1 takes into account the maximum wind speed (Wind V. max), Model-2 the frequency of calm wind days. The two punctuation points indicate the interaction between the variables. Significance levels: $p \leq 0.5$ (.), $p \leq 0.1$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***)).

	<i>Statistical model</i>	<i>Estimates</i>	<i>Std. Error</i>	<i>t value</i>	<i>Pr(>t)</i>
	Model-1				
Cupressaceae	Wind direction mean NW	-3.26	1.1	-3.06	0.002 **
	Wind direction mean NW : Wind V. max	3.12	0.1	3.23	0.0008 ***
	Model-2				
	Wind direction mean SE	0.68	0.32	2.09	0.03 *
	Model-1				
Pinaceae	Wind V. max	0.11	0.003	2.80	0.005 **
	Wind direction mean N : Wind V. max	0.06	0.07	2.04	0.04 *
	Wind direction mean NE : Wind V. max	0.07	0.005	3.12	0.001 **
	Wind direction mean E : Wind V. max	0.09	0.05	3.72	0.0002 ***
	Wind direction mean SE : Wind V. max	0.11	0.06	3.42	0.0006 ***
	Wind direction mean S : Wind V. max	0.11	0.07	3.34	0.0008 ***
	Wind direction mean SW : Wind V. max	0.08	0.08	2.34	0.01 *
	Model-2				
	Wind direction mean W	0.69	0.39	1.75	0.06 .
	Wind direction mean S : Wind calm	- 0.97	0.56	- 1.73	0.08 .
	Model-1				
Castanea	Wind direction mean N	3.34	1.15	2.89	0.004 **
	Wind direction mean S	- 1.54	0.66	- 2.33	0.002 **
	Wind direction mean N : Wind V. max	- 0.26	0.12	- 2.11	0.03 *
	Model-2				
	Wind direction mean N	0.86	0.41	2.09	0.03 *
	Model-1				
Olea	Wind direction mean NW	- 1.36	0.29	- 2.64	0.009 **
	Wind V. max	- 0.16	0.07	- 2.46	0.01 *
	Wind direction mean NW : Wind V. max	1.84	0.68	2.60	0.008 **
	Model-2				
	Wind direction mean N	2.27	0.21	3.58	0.009 **
	Wind direction mean NE : Wind calm	0.39	0.19	2.05	0.04 *
	Model-1				
Quercus	Wind direction mean NE : Wind V. max	0.61	0.34	1.94	0.03 *
	Wind direction mean NW : Wind V. max	0.22	0.12	- 1.66	0.08 .
	Wind direction mean S : Wind V. max	0.07	0.06	1.77	0.06 .
	Model-2				
	Wind direction mean N : Wind calm	- 2.60	1.11	- 2.31	0.02 *



7. Allergenic airborne pollen in Florence (Italy): a record of 22 years.

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Abstract

The allergenic airborne pollen in Florence was recorded by analyzing sixteen moss cushions sampled at the same site during three surveys since 1995. The choice of the moss cushions is due to their natural capacity to accumulate pollen grains for a prolonged time interval. The allergenic taxa considered in our analysis are Cupressaceae, Poaceae, and *Parietaria* sp. The results reveal a constant dominance of Cupressaceae pollen grains, which constitute more than half of the total pollen content in each year of sampling, although changes in the quantity were recorded. The high concentrations of these pollen grains reflect the great number of these plants in the city. Starting from 2008, a change in the amount of the herbaceous pollen grains is observed, with a strong increase of *Parietaria* and a decrease of Poaceae pollen. This can be related to the change in the street cleaning method, performed during the central time interval of our analysis. The results of this work suggest how much the composition of public green and the management of public roads influence the allergenic airborne pollen in urban areas.

Keywords: palynology; airborne pollen; allergy; moss cushions.

1. Introduction

Air quality, one of the most important determinants of health, is constantly monitored for both chemical and biological components. Among the latter, airborne pollen grains have perhaps the most remarkable impact on public health, since pollinosis affects about 40% of the European population (D'Amato et al. 2007). The effects of pollinosis are also greater in urban areas where atmospheric pollution enhances the negative effects on allergic subjects (Bosh-Cano et al. 2011). In addition, the use of allergenic plants in the green spaces and along the urban avenues increases the risk of respiratory disease (Carinanos and Casares-Porcel 2011).

The aim of this study is to detect the allergenic airborne pollen (AAP) in the city of Florence (Italy) and its changes during the last 22 years, thanks to the analyses of moss cushions sampled at the same sites three times starting from 1995. The use of moss cushions is justified by their high capacity to collect and preserve pollen grains for a prolonged time. According to literature, the span of time lasting from two to fifteen years of pollen rain (Cowder and Cuddy 1973; Caseldine 1981; Bradshaw 1981; Cundill 1991; Mulder and Janssen 1998; Ciani et al. 2019). Therefore, the pollen content of the cushions provides a global view of the pollen rain which reduces the effects of the annual variations.

2. Materials and methods

2.1 Study site

The city of Florence (Italy) (43°47'14"64 N, 11°14'59"64 E, 50 m a.s.l.) (Figure 1) is located in north-eastern Tuscany, in the middle part of the Arno River basin. The urban area is bordered to the north-east and to the South by hill chains of medium-low altitude (NE: up to 934 m a.s.l.; S: up to 310 m a.s.l.).

The climate is sub-Mediterranean, with dry and hot summers, mild winters and wet autumns and springs (Petralli et al. 2011). The average annual temperature is 15.5° C, while the annual average rainfall is around 830 mm (Ximenian Observatory of Florence 2005). In the plain, the only forested area can be considered the floodplain woods of Cascine Park where large grassland areas were also

found. Small patches dominated by herbaceous plants are the flower beds, characterized by many cultivated species, often of alien origin, and the marginal-ruderal species growing along the roads. Spontaneous vegetation of the municipal territory is also represented by semi-natural riparian vegetation along the Arno river and its tributaries. The hills surrounding Florence are mainly characterized by the classical Tuscan landscape up to an elevation of about 300 m a.s.l.: vineyards and olive groves alternate with oak woods (*Quercus pubescens* Will.) and cypress plantations. In some areas, little patches of grasslands, in large parts in way of abandonment, can find.

2.2 Analyses on moss cushions

Sixteen moss cushions were collected at ground level in the city center. The sampling was made at the same points in the years 1995, 2008 and 2017. The sampling sites are shown in Figure 1.

All the samples were treated using routine methodologies, including treatments with HCl, HF, sodium hexametaphosphate, NaOH and the acetolytic method (Erdtman 1960), and observed at light microscopy at 40 x and 63 x magnification. Pollen concentrations are expressed as absolute pollen frequency (APF, numbers of grains per gram of sample).

The allergenic component of the pollen rain studied over the years including both arboreal and herbaceous plants. Among the arboreal, Cupressaceae pollen grains have the highest potential of pollinosis. Among the herbaceous plants, our analysis took into account the family of Poaceae and the genus *Parietaria* sp., the main allergenic genus of the Urticaceae family.

In this work, the amount of AAP is therefore represented by the sum of the absolute pollen frequencies of Cupressaceae, Poaceae and *Parietaria* revealed in each sample and by the quantitative variation of these taxa over time.

3. Results

The sixteen moss cushions collected during 1995 showed a mean AAP absolute frequency of about 15000 grains/gram. The most represented taxon was the family of Cupressaceae. Poaceae pollen grains were the second most represented taxon, followed by *Parietaria* pollen grains (Figure 2).

In the moss samples of 2008, the mean AAP absolute frequency was about 19000 grains/gram. Pollen grains of Cupressaceae family were the most represented followed by *Parietaria* and Poaceae, which displayed similar values (Figure 2).

The moss samples collected in the year 2017 showed a mean AAP absolute frequency of 18500 grains/gram. Cupressaceae was the most represented taxa, followed by *Parietaria* and Poaceae (Figure 2).

4. Discussion and conclusion

The analysis of the moss cushions shows a fairly clear image of the allergenic airborne pollen in the city of Florence. In the period under consideration, allergenic pollen grains reach extremely high values, around 15000 – 19000 grains per gram of sample. The year 2008 records, in particular, an increase in these values and a noticeable change in the AAP composition.

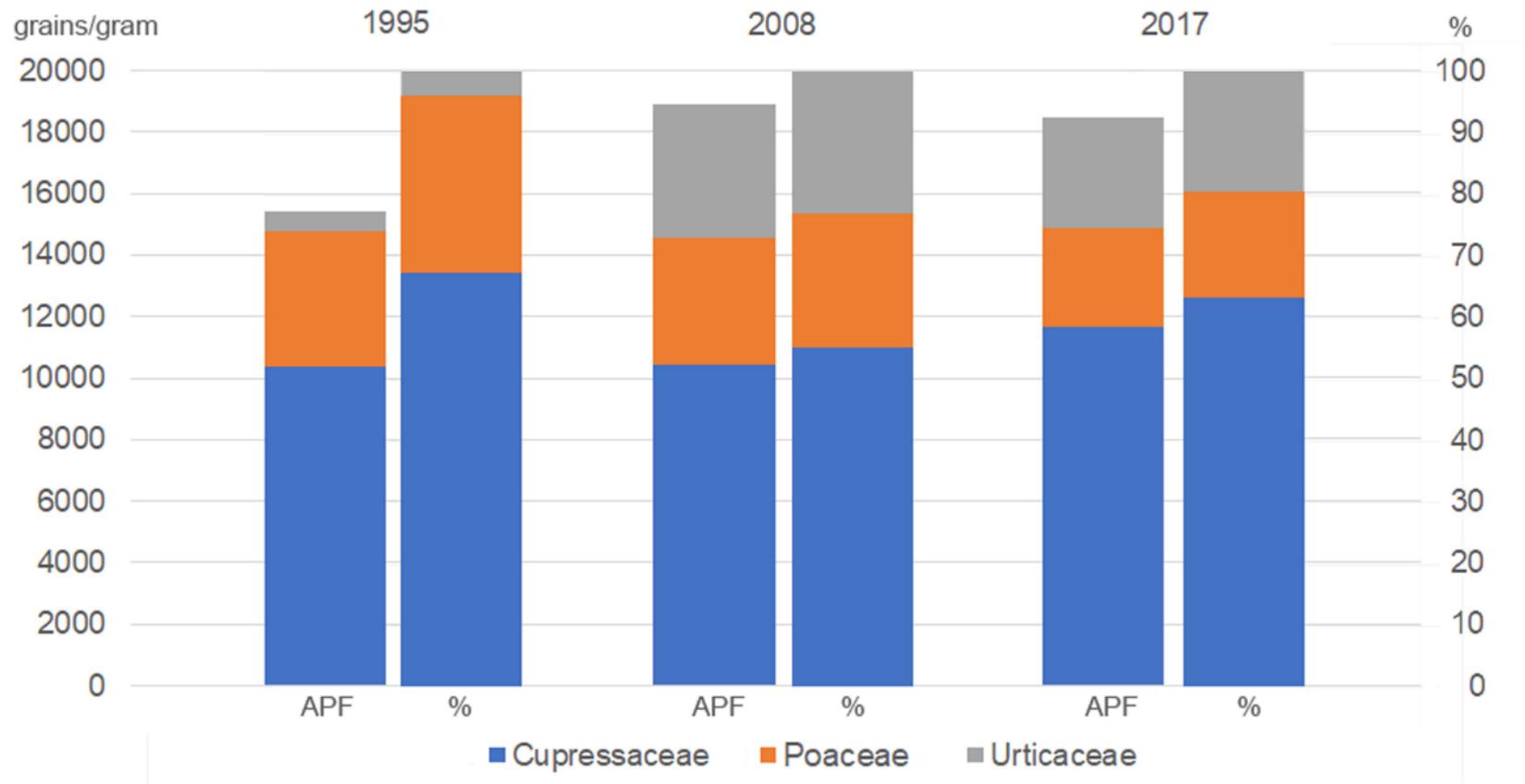
Over the years, Cupressaceae pollen grains dominate the pollen spectra, always exceeding half of the total AAP. The pollen grains of this family are one of the most common causes of pollinosis in many Mediterranean countries such as France, Israel, and Spain, as also in other parts of the world. Cupressaceae pollen grains dominate the pollen spectra for a large part of the year, due both to their mass flowering and the prolonged pollen season of different species (Diaz de la Guardia et al. 2006). The same situation is observed in the city of Florence, where Cupressaceae trees are mainly widespread for ornamental use and the second plant's type used in the urban green areas. The rise of Cupressaceae pollen during the last ten years may be related to the recent increase in the number of trees (Municipality of Florence, Territorial Information System 2010, 2016).

Grass pollen represents an important percentage of AAP in the city of Florence. In our analyses, it shows a linear decrease during the time interval considered. In 1995 is the second most important taxa, reaching 29% of the total AAP. Its value decreases to 22% in 2008 and 17% in 2017. A general decrease in grass pollen has been observed in numerous European cities and has been related to the reduction of grassland observed in many countries (D'Amato et al. 2007). However, grass pollen continues to be the main cause of allergy in many parts of the world due to its high allergenic potential. *Parietaria* has relevant etiologic importance because of its large diffusion in many Mediterranean countries and the prolonged pollen production during the year. In Florence, this plant is widespread, in particular at the foot of the buildings and on the walls and along the Arno river and its tributaries. In our analysis, *Parietaria* pollen grains show a substantial increase starting from 2008, with percentages of about 20% or more, exceeding Poaceae in 2017. This can be related to the different management of street sweeping implemented by the municipality of Florence during the central time interval of our analysis that led to a general increase in herbaceous plants along the sidewalks. In conclusion, a change in the composition of the allergenic pollen rain is easily visible in Florence during the last twenty years and results particularly evident for the grains of the herbaceous plants. The variation seems to be mainly related to human activities. The rise of Cupressaceae pollen may be a direct consequence of the increase of these trees in the municipality; that of Urticaceae of the management of urban streets.

Figure 1: A) Geographical location of Florence and B) position of the sampling points in the city center



Figure 2: APF (grains/gram) and percentages of the AAP recorded in each year of sampling





8. Airborne herbaceous pollen in Florence (Italy)

(preliminary results)

*Cover image: The north bank of the Arno
River from the Cascine Park*

1. Introduction

Over the last years, numerous studies have been devoted to the seasonal variations of airborne pollen concentrations, focusing in particular on those with the highest allergenic potential (Frenguelli et al. 1989; 1991). These studies have remarkable importance in urban areas, where more than half of the world's population lives (United Nations 2004). Since pollen concentration may vary even on a strictly local scale (Kasprzyk 2010), monitoring should be carried out in several places in the cities, to overcome the limitation due to the use of a single sampling point.

In this paper are reported the airborne herbaceous pollen concentrations detected in Florence (Italy), focusing in particular on the most represented families (Poaceae and Urticaceae). The comparison with pollen data of previous years allows evaluating the temporal variation reached by pollen concentrations in different sites inside the historical city center.

2. Materials and methods

Florence (43°47'14.64" N, 11°14'59,64" E, 50 m a.s.l.) (Figure 1) is located in north-eastern Tuscany, the central part of the Arno river valley, which crosses the city. The climate is continental temperate; the average annual temperature is 15.5° C and the average annual rainfall is around 830 mm.

The urban green areas of the city are represented by public/private parks and gardens where trees and shrubs are cultivated. The banks of the Arno River host numerous ruderal and nitrophilous herbs (Foggi et al 2008). Rural areas and semi-natural woodlands (mainly *Quercus*-dominated) are scattered in the territory surrounding the town.

The aerobiological study was performed by means of a Hirst-type volumetric spore trap (Hirst 1952) located in the city center, on the roof of the Department of Biology of the University of Florence (Bio-UNIFI). The data from this sampling station refer to the time interval of September 2018 – October 2019.

In order to detect the temporal and quantitative variations, the pollen concentrations recorded by the Bio-UNIFI sampler were compared with the data coming from the volumetric sampler of ARPAT

(Regional Agency for Environmental Protection of Tuscany) located in the western part of the city (Figure 1), about 4km away from the Bio-UNIFI sampler. ARPAT pollen data refer to the time intervals 2009 - 2018 and were freely downloaded from the website <http://www.arpat.toscana.it>. Data relative to 2019 are not available.

The main pollen season (MPS) was calculated using the Andersen method (1991) with the ‘AeRobiology’ package (Rojo et al. 2018) on R-Studio software (R Core Team 2018).

The landscape surroundings both the volumetric sampler were studied in a buffer of 1km in radius to detect the proximity effect of the sources to the pollen data

3. Results and discussion

The comparison between the pollen data of the two sampling stations allowed to evaluate the temporal and spatial variations of herbaceous airborne pollen on Florence.

Bio-UNIFI pollen monitoring during 2019 revealed a substantial advance and extension of Poaceae MPS with respect to the average of the ten previous years monitored by ARPAT (Table I and Figure 2). The earlier beginning of Poaceae pollen season was already observed during the last years in other Italian cities (Frenguelli 2002; Cristofolini et al. 2019), as in other Mediterranean countries (Galan and Dominguez-Viches 2012). The total pollen sum of Poaceae from Bio-UNIFI sampler is quite similar to the ten previous years monitored by ARPAT (Table I) suggesting that the landscape surrounding the two traps does not considerably influence the airborne pollen amounts of these plants (Table II and Figure 1). Despite the strictly local influence of pollen sources (Ciani et al. 2019), it is extremely difficult to identify the precise origin of the Poaceae grains due to the wide range of habitats where these plants grow (Smith et al. 2005). In general, Poaceae total pollen sums appeared scarce in both the volumetric sampler datasets with respect to studies conducted in central Italy in the past (Spiekma et al. 1989). In recent years, Poaceae values in Florence are similar to those recorded both in Italy and in other European countries (Peternel et al. 2006). Despite seasonal and annual variations

(Spieksma et al. 1989), the pollen concentrations show a substantial decrease in the last years (Ugolotti et al. 2015).

Urticaceae MPS beginning showed a delay of about ten days with respect to the mean of ten previous years monitored by ARPAT, in addition to a considerable decrease of the total pollen sum (Table I and Figure 2). This result is in contrast with the records of other Italian monitoring stations (Cristofolini et al. 2019) and other Mediterranean regions like Spain (Cariñanos et al. 2004) or Greece (Fotiou et al. 2011).

The greatest difference between the two sampling sites can be observed from a quantitative point of view. The mean of the total pollen sum during the ten previous years is greater by more than 2000 grains/m³ compared to the Bio-UNIFI survey of 2019 (Table I). The area surrounding the Bio-UNIFI sampler (the historical city center of Florence) display a higher percentage of built-up areas with respect to the ARPAT sampler (Table II). This evidence does not support the low concentration of pollen grains of ruderal plants such as Urticaceae. The explanation of this data could be found in the management of urban streets, probably more accurate in the city center than in the periphery.

Acknowledgments

This study was possible thanks to the opportunity to use the Lanzoni VPPS 2000 of the Department of Environmental and Life Sciences of the University of Genoa, courtesy of Prof. Montanari.

Figure 1: Geographical location of Florence and of the Bio-UNIFI (red dot) and ARPAT (purple square) samplers.



Figure 2: Trend plot for Poaceae and Urticaceae pollen concentrations recorded by Bio-UNIFI (red lines) and ARPAT (black lines) samplers. Vertical dotted lines refer to the beginning/end of the pollen season. ARPAT pollen values refers to the average daily concentrations recorded during the time interval 2009-2018

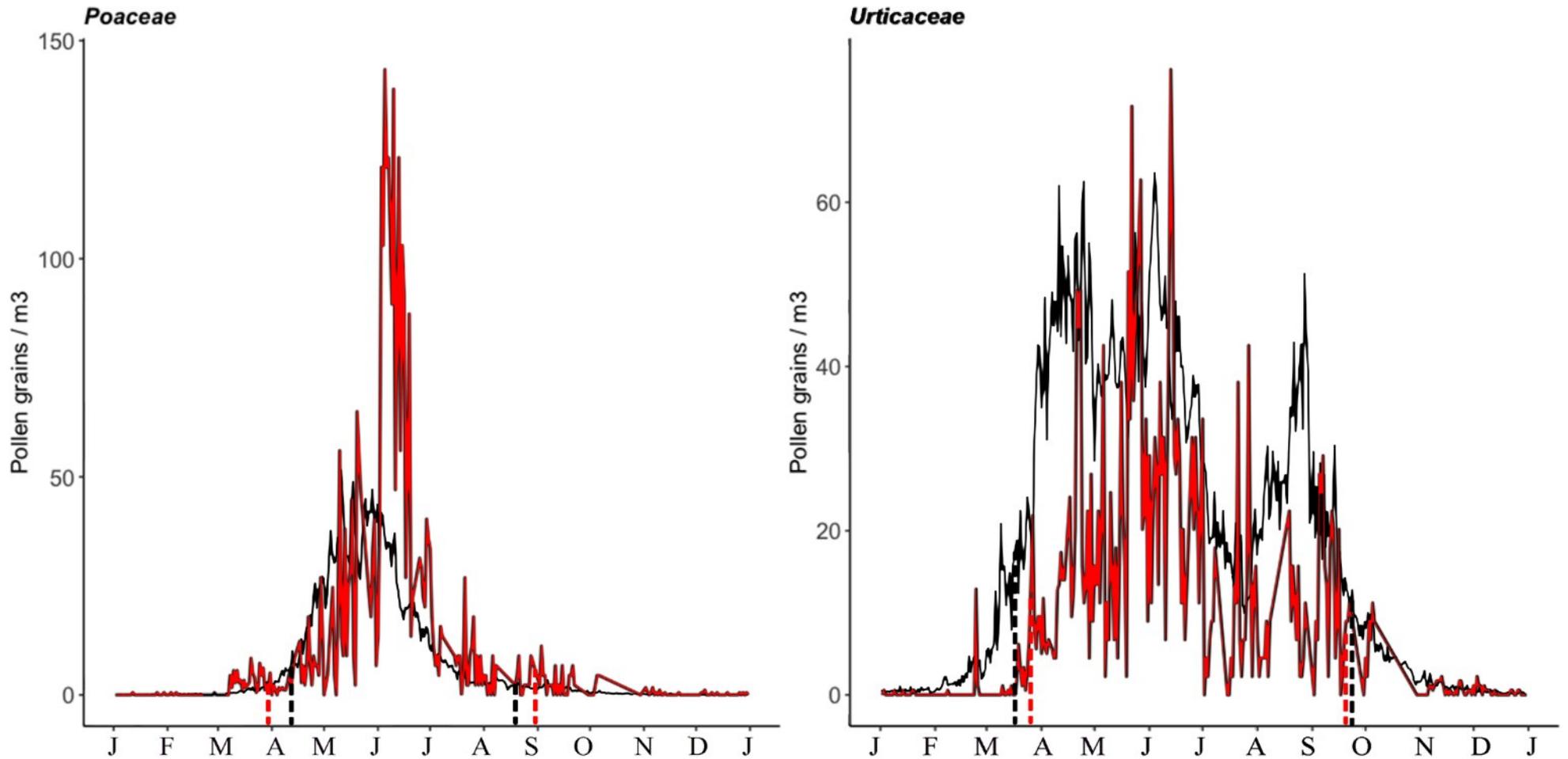


Table I: Airborne pollen data recorded by Bio-UNIFI and ARPAT samplers. Total pollen sums and peaks values are expressed as pollen grains/m³.

	<i>Main pollen season – (MPS)</i>	<i>MPS length (days)</i>	<i>Total pollen sum</i>	<i>Peak value –peak date</i>
Poaceae				
Bio-UNIFI 2019	31/03 – 31/08	154	3510	143 – 05/06
ARPAT 2009-2018	11/04 – 17/08	129	3081	122 – 24/06
Urticaceae				
Bio-UNIFI 2019	27/03 – 23/09	181	3191	76 – 13/06
ARPAT 2009-2018	17/03 – 25/09	193	5025	110 – 26/05

Table II: Land cover of the 1 km radius buffers surrounding the volumetric traps.

	Bio-UNIFI sampler		ARPAT sampler	
	<i>m</i> ²	%	<i>m</i> ²	%
Built-up areas	2,742,461	87.3	2,265,048	72.1
Mixed woodlands	182,049	5.8	283,555	9
Shrublands	33,274	0.9	48,299	1.5
Crops	-	-	291,920	9.3
Water bodies	181,195	5.8	8,103	0.3
Olive groves	3,636	0.2	245,912	7.8

9. Conclusions

The study of the pollen rain recorded both at ground and roof level using two different methodologies, gravimetric (moss cushions) and volumetric sampling, has provided an accurate image of the airborne pollen on the city of Florence, an urban settlement of central Mediterranean area.

The results show that the pollen rain recorded in the urban area is relatively similar in both moss cushions and volumetric sampling data, with noticeable differences that can be observed only from a quantitative point of view. The two types of samplers operate at different spatiotemporal scales and can be used in parallel providing data that integrate with each other: the volumetric sampler evidences temporal changes and fluctuations on the background of the moss cushions record. This study confirms that the volumetric sampler is more useful for allergological studies to safeguard public health thanks to the temporal precision of the pollen records. The use of moss cushion is instead particularly useful for broad-spectrum surveys and the study of the relationship between flora and pollen rain and well reflects the surrounding environmental context. The results of my study show that pollen content from moss samples covers a period greater than five years, ensuring the continuous pollen deposition and, as a consequence, attenuating the different annual rates of pollen production.

From a qualitative point of view, the pollen content of moss cushions well reveals the influence of the urban trees composition which is appreciable at different distance depending on the species. The records display a strong prevalence of arboreal pollen with a slightly tendency to increase. The dominance is due to the high number of ornamental trees of the urban green areas in addition to the plants growing on the hilly slopes facing the city.

The interpolation of the data recorded in the sampling sites inside the city shows that pollen accumulation is uneven across the city and the values recorded at ground level indicate a strong influence of the near urban green areas, both from a qualitative (most represented trees in the city) and quantitative point of view (direct correlation between pollen amount and the number of trees in the surroundings).

Among the ornamental trees, cypresses are widespread inside the city and in extra-urban areas and their pollen grains dominate the aerobiological record of Florence. Cupressaceae pollen always displays higher values in the volumetric sampler record than in the moss cushions: the lower amount could be due to the scarce preservation of these pollen grains and to the shielding effect of the urban buildings on the airborne pollen diffusion. Despite these features, the proximity effects of these plants to the pollen records are also considerable in a short area. These results underline the importance of recording the pollen concentrations in several sites inside the urban contexts, notably at the ground level, to determine the local actual risk factors for people with pollinosis syndromes.

The highest herbaceous pollen concentrations are localized in the proximity of the Arno River where numerous ruderal plants and small patches of the natural vegetation have been found. The impact of these areas is highlighted by the strong correlation between herbaceous pollen concentrations and the nearness of the sources. Poaceae dominates the herbaceous pollen spectra, although a substantial decrease in recent years was observed in Florence as in other European cities. The opposite trend is observed for Urticaceae pollen grains, in particular for *Parietaria*. The rise of these pollen grains may be linked to the change in streets sweeping, suggesting the importance of urban management not only of green areas but also of built-up areas. These evidences, detected at ground level by the gravimetric sampling, have been confirmed by the volumetric monitoring carried out in the last year using the traps located at roof level.

Regarding the extra-urban pollen contribution, this study shows that the jointed analysis between pollen values and wind data, combined with land-cover analysis, can provide a valuable tool for understanding the dynamics of pollen transport and to evaluate the actual pollen contribution from the area surrounding the city. The greatest pollen contribution in Florence is probably provided by the vegetation of the Northern reliefs. This is particularly evident for Cupressaceae and *Quercus* pollen grains when the wind blows at high intensity. The local contribution from nearby pollen sources is well appreciable in periods of calm wind for Cupressaceae and *Olea*.

These investigations furnish a basis for further researches, based on the information obtained over the years through the new volumetric pollen trap located in the center of the city. The importance of a network of traps at ground level is crucial in urban environments for green space planning and management in order to ensure the citizen's wellness.

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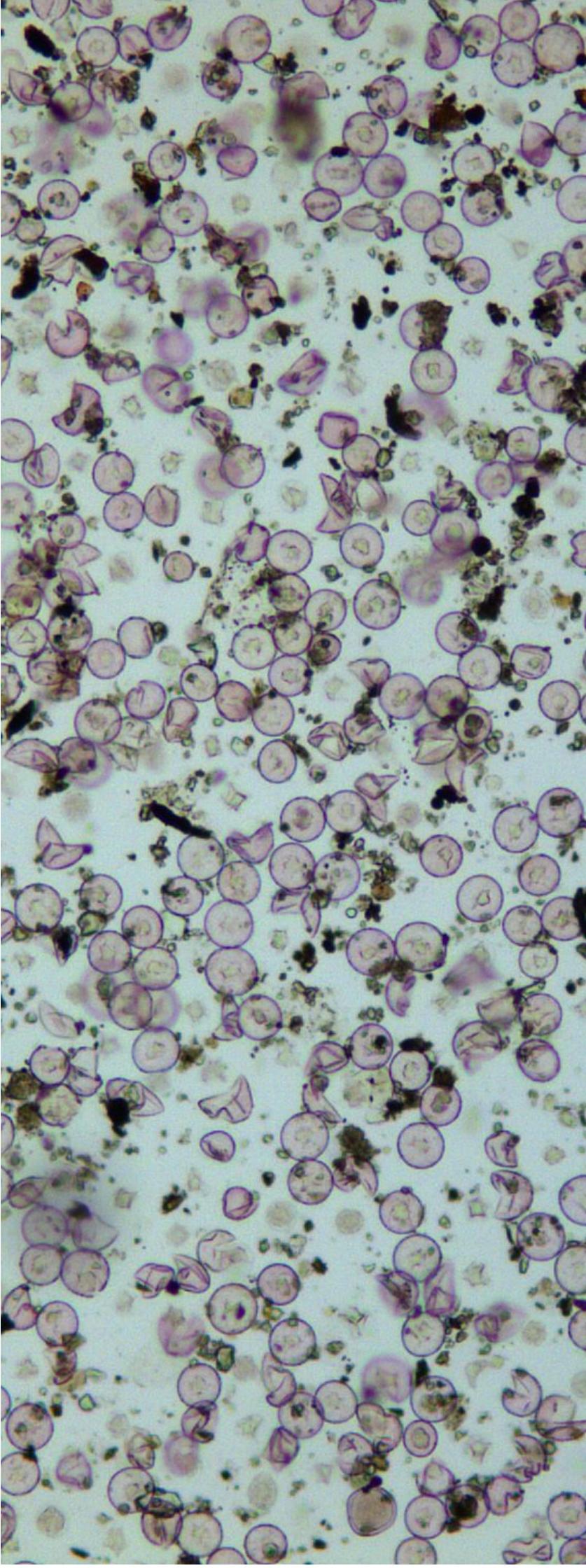
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11. Ph.D. activities

Francesco Ciani

**Ph.D. Course: “Evolutionary
biology and ecology”**

Cycle XXXII

Supervisor
Dott. Foggi Bruno

Co-Supervisor
Prof.ssa Mariotti Lippi Marta

Publications

Giuliani C., Ciani F., Gonnelli T., Benvenuti M., Pallecchi P., Aranguren B., Revedin A., Mariotti Lippi M. 2015. *Palynology in the Paleolithic site of Poggetti Vecchi (GR)*. In: Mediterranean Palynology Symposium, GPSBI-APPLE, Roma, 8-10 settembre 2015, Aracne, p. 57, ISBN:978-88-548-8693-3.

Mariotti Lippi M., Ciani F., Florenzano A., Torri P., Montecchi M.C., Landi Degl'Innocenti V., Ricciardo D., Rattighieri E., Mercuri A.M. 2017. *Vitis representation in modern pollen spectra from Southern Europe vineyards*. In: Mediterranean Palynology Symposium 2017, Barcelona, 4-6 September 2017, Concepción De Linares and Jordina Belmonte, p. 117, ISBN:9788494537875.

Benvenuti M., Bahain J.J., Capalbo C., Capretti C., Ciani F., D'Amico C., Esu D., Giachi G., Giuliani C., Gliozzi E., Lazzeri S., Macchioni N., Mariotti Lippi M., Masini F., Mazza P.A., Pallecchi P., Revedin A., Savorelli A.; Spadi M., Sozzi L., Vietti A., Voltaggio M., Aranguren B. 2017. *Paleoenvironmental context of the early Neanderthals of Poggetti Vecchi for the late middle Pleistocene of Central Italy*. Quaternary Research, vol. 88, pp. 327-344, ISSN:0033-5894. DOI: <https://doi.org/10.1017/qua.2017.51>

Ciani F., Attolini D., Bellini C., Mori Secci M., Gonnelli T., Pallecchi P., Mariotti Lippi M. 2018. *Archeobotanical investigations in the ancient city of Gonfienti, Italy (Bronze Age, Iron Age)*. In: 2018 IEEE International Conference on Metrology for Archaeology and Cultural Heritage – MetroArcheo 2018, Cassino, Italia, October 22-24, 2018, Institute of Electrical and Electronics Engineers, vol. IEEE Catalog Number: CFP18O73-USB, pp. 489-493, ISBN:978-1-5386-5275-6.

Aranguren B., Grimaldi S., Benvenuti M., Capalbo C., Cavanna F., Cavulli F., Ciani F., Comencini G., Giuliani C., Grandinetti G., Mariotti Lippi M., Masini F., Mazza P.A., Pallecchi P., Santaniello F., Savorelli A., Revedin A. 2019. *Poggetti Vecchi (Tuscany, Italy): A late Middle Pleistocene case of human–elephant interaction*. Journal of Human Evolution, vol. 133, pp. 32-60, ISSN:0047-2484 DOI: <https://doi.org/10.1016/j.jhevol.2019.05.013>

Ciani F., Attolini D., Bellini C., Pallecchi P., Mariotti Lippi M. 2019. *Pollen analysis in the early Middle Ages Florence (Italy)*. In: 18th Conference of the International Workgroup for Palaeoethnobotany, Lecce, Italia, 3 - 8 June 2019, Università del Salento, p. 48, ISBN:978-88-8305-146-3.

Ciani F., Marchi M.G., Dell'Olmo L., Mariotti Lippi M., Foggi B. 2019. “*Effect of land cover and wind on airborne Olea pollen*”. In: Desprat S., Daniau A., Sánchez Goñi M. The Mediterranean Palynological Societies Symposium 2019. Abstract book. MedPalyno 2019, Jul 2019, Bordeaux, France. Université de Bordeaux, p. 15, ISBN 978-2-9562881-3-8. [Hal-02274992](https://hal.archives-ouvertes.fr/hal-02274992)

Ciani F., Mariotti Lippi M., Foggi B. 2019. “*Impact of ornamental urban trees on the pollen records in the city of Florence (Italy)*”. In: Desprat S., Daniau A., Sánchez Goñi M. The Mediterranean

Palynological Societies Symposium 2019. Abstract book. MedPalyno 2019, Jul 2019, Bordeaux, France. Université de Bordeaux, p. 16, ISBN 978-2-9562881-3-8. [Hal-02274992](#)

Ciani F., Cirilli O., Pandolfi L., Bartolini Lucenti S., Savorelli A., Sforzi A., Rook L., Mariotti Lippi M. 2019. “*Pollen analysis on spotted hyaena (Crocuta crocuta) coprolites from the fossiliferous site of Buca della Jena, Southern Tuscany (Roselle, Grosseto, Italy).*” In: Desprat S., Daniau A., Sánchez Goñi M. The Mediterranean Palynological Societies Symposium 2019. Abstract book. MedPalyno 2019, Jul 2019, Bordeaux, France. Université de Bordeaux, p. 76, ISBN 978-2-9562881-3-8. [Hal-02274992](#)

Ciani F., Pampaloni M., Foggi B., Mariotti Lippi M. *Comparing pollen data from moss cushions and a volumetric sampler: the study in the city of Florence (Italy).* 2019. Grana. (in press)

Ciani F., Foggi B., Mariotti Lippi M. *Cupressaceae pollen: new data about diffusion, record and preservation.* 2020. Plant Biosystems. DOI: [10.1080/11263504.2020.1727982](#)

Courses and interdisciplinary education

15/02-16/02/2017. Aggiornamento metodiche di laboratorio. Prof.ssa A. M. Mercuri, Dr.ssa P. Torri. Dipartimento di Scienze della Vita sede, Università degli studi di Modena e Reggio Emilia.

24/02-12/06/2017. Geologia. Prof. E. Pandeli. Corso di Laurea triennale in Scienze Naturali. Università degli Studi di Firenze.

25/02-14/06/2017. Guida all'utilizzo del software QGis, in Scienza della vegetazione e conservazione e gestione delle risorse vegetali. L. Dell'Olmo. Corso di Laurea magistrale in Scienze della Natura e dell'Uomo. Università degli Studi di Firenze.

01/03-16/06/2017. Laboratorio di Ecologia vegetale. Dr. B. Foggi. Corso di Laurea magistrale in Scienze della Natura e dell'Uomo. Università degli Studi di Firenze.

04/2017. Writing for research. Dr. R. Lynch. Centro Linguistico d'Ateneo, Università degli Studi di Firenze.

03/05-10/05/2017. Attività di campo multidisciplinare, Isola d'Elba. Dr. B. Foggi, Prof. E. Pandeli, Dr. L. Dapporto. Corso di Laurea triennale in Scienze Naturali. Università degli Studi di Firenze.

02/10-14/12/2017. Modern regression methods. Prof. P. Šmilauer, Quantitative Ecology module. Faculty of Science, University of South Bohemia.

02/10-14/12/2017. Creative publishing in community ecology. Prof. J. Lepš, F. de Bello, L. Goetzenberger, J. Hrcek. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

02/10-14/12/2017. Design and analysis of ecological experiments. Prof. J. Lepš, P. Šmilauer. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

02/10-14/12/2017. Community ecology. Prof. J. Lepš, V. Novotný. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

02/10-14/12/2017. Evolutionary Ecology. Prof. D. Boukal. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

02/10-14/12/2017. Ecology of biological invasions. Prof. K. Prach. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

02/10-14/12/2017. Functional traits in ecology. Prof. F. de Bello. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

25/02-14/06/2018. Conservazione e gestione delle risorse naturali. Dr. B. Foggi. Corso di Laurea magistrale in Scienze della Natura e dell'Uomo. Università degli Studi di Firenze.

25/02-14/06/2018. Invasioni biologiche. Prof.ssa F. Scapini. Corso di Laurea magistrale in Scienze della Natura e dell'Uomo. Università degli Studi di Firenze.

04/2018. Botanica applicata e riconoscimento delle piante. Dr. B. Foggi. Master di II livello in Progettazione paesaggistica. Dipartimento di Architettura, Università degli Studi di Firenze.

04/2018. Tutela delle Risorse Vegetali. Dr. B. Foggi. Master di II livello in Progettazione paesaggistica. Dipartimento di Architettura, Università degli Studi di Firenze.

17/09-14/12/2018. Scienza della vegetazione e conservazione e gestione delle risorse vegetali. Prof. D. Viciani. Corso di Laurea magistrale in Scienze della Natura e dell'Uomo. Università degli Studi di Firenze.

17/09-19/12/2018. Progettazione delle aree verdi. Prof. B. Guccione. Corso di Laurea triennale in Scienze vivaistiche, ambiente e gestione del verde. Università degli Studi di Firenze.

25/02-14/06/2019. Botanica Forense. Prof.ssa M. Mariotti. Corso di Laurea magistrale in Biologia molecolare ed applicata. Università degli Studi di Firenze.

03/05-31/05/2019. Corso di Statistica ed introduzione all'utilizzo di R. Prof. G. Santini. Offerta didattica per dottorandi. Dipartimento di Biologia, Università degli Studi di Firenze.

15/05-24/05/2018. Attività di campo multidisciplinare, Gerfalco. Dr. B. Foggi, Prof. E. Pandeli, Dr. L. Dapporto. Corso di Laurea triennale in Scienze Naturali. Università degli Studi di Firenze.

23/05-31/05/2019. Attività di campo multidisciplinare, Monte Carpegna. Dr. B. Foggi, Prof. E. Pandeli, Dr. L. Dapporto. Corso di Laurea triennale in Scienze Naturali. Università degli Studi di Firenze.

26/08-31/08/2019. Advanced Aerobiology Course on automatic and real-time pollen monitoring. Prof. J. Buters, B. Clot, B. Crouzy, T. Könemann, D. O'Connor, B. Sikoparija, C. Skjoth, M. Sofiev, F. Tummon. Federal Office of Meteorology and Climatology MeteoSwiss.

Congresses attendance

4-6/9/2017. Medpalyno 2017, The Mediterranean Palynological Societies Symposium 2017 (Barcellona). Comunicazione orale: Mariotti Lippi M., Ciani F., Florenzano A., Torri P., Montecchi M.C., Landi Degl'Innocenti V., Ricciardo D., Rattighieri E., Mercuri A.M., 2017. "*Vitis representation in modern pollen spectra from Southern Europe vineyards*". Medpalyno 2017 Abstract Book, p. 117. ISBN: 978-84-945378-7-5 (Book). ISBN: 978-84-945378-8-2 (e-Book).

20-23/9/2017. 112° Congresso della Società Botanica Italiana - SBI (Parma). Poster: Ciani F., Giuliani C., Foggi B., Giachi G., Aranguren B., Mariotti Lippi M. "*The site of Poggetti Vecchi (GR): a paleopalynology research*". 112° Congresso SBI Abstract Book, p. 126. ISBN 978-88-85915-21-3.

26-28/2/2018. 14° Conference of Environmental Archaeology 2018. Poster: Ciani F., Dell'Olmo L., Mariotti Lippi M., Foggi B. "*Land cover and land use change in the archaeological sites of the Prato province (Tuscany, Italy)*". CEA 2018 Abstracts book, pp. 173-175. ISBN: 978-88-943442-0-2.
27/02/2018. Chairman at Session 6: "Reconstructing past landscape: flora insights from archaeological sites."

3-7/9/2018. 11° International Congress on Aerobiology 2018 (Parma). Comunicazione orale: Ciani F., Pampaloni M., Mori Secci M., Begliomini V., Gonnelli T., Foggi B., Mariotti Lippi M. "*Allergenic airborne pollen in Florence (Italy)*". ICA 2018 Abstract book, p. 89.

22-24/10/2018. IEEE International Conference on Metrology for Archaeology and Cultural Heritage – MetroArcheo 2018 (Cassino). Comunicazione orale: Ciani F., Attolini D., Bellini C., Mori Secci M., Gonnelli T., Pallecchi P., Mariotti Lippi M. "*Archeobotanical investigations in the ancient city*".

of Gonfienti, Italy (Bronze Age, Iron Age)”. MetroArcheo 2018 proceedings, pp. 489-493. ISBN: 978-1-5386-5275-6.

16/3/2019. Conferenza “Paleostorie di Maremma: il sito fossilifero di Buca della Jena”. Comunicazione orale: Ciani F., Mariotti Lippi M. “Analisi polliniche su coproliti di iena maculata (*Crocota crocuta*) dal sito fossilifero di Buca della Jena (Roselle, Grosseto, Italia).” Museo di Storia Naturale della Maremma. Grosseto.

3-8/6/2019. 18th Conference of the International Workgroup for Palaeoethnobotany – IWGP 2019 (Lecce). Poster: Ciani F., Attolini D., Bellini C., Pallecchi P., Mariotti Lippi M. “*Pollen analysis in the Early Middle Ages Florence (Italy)*”. IWGP 2019 proceedings, p. 48. ISBN: 978-88-8305-146-3.

9-11/7/2019. Medpalyno 2019, The Mediterranean Palynological Societies Symposium 2019 (Bordeaux).

Poster - 1: Ciani F., Marchi M.G., Dell’Olmo L., Mariotti Lippi M., Foggi B. “*Effect of land cover and wind on airborne Olea pollen*”. Medpalyno 2019 Abstract Book, p. 15. ISBN: 978-2-9562881-3-8.

Poster – 2: Ciani F., Mariotti Lippi M., Foggi B. “*Impact of ornamental urban trees on the pollen records in the city of Florence (Italy)*.” Medpalyno 2019 Abstract Book, p. 16. ISBN: 978-2-9562881-3-8.

Poster – 3: Ciani F., Cirilli O., Pandolfi L., Bartolini Lucenti S., Savorelli A., Sforzi A., Rook L., Mariotti Lippi M. “*Pollen analysis on spotted hyaena (*Crocota crocuta*) coprolites from the fossiliferous site of Buca della Jena, Southern Tuscany (Roselle, Grosseto, Italy)*.” Medpalyno 2019 Abstract Book, p. 76. ISBN: 978-2-9562881-3-8.

4-7/9/2019. 114° Congresso Società Botanica Italiana - SBI (Padova). Poster: Ciani F., Marchi M.G., Dell’Olmo L., Foggi B., Mariotti Lippi M. “*The effect of wind and land cover on the diffusion of Quercus pollen*.” 114° Congresso SBI Abstract Book, p. 62. ISBN 978-88-85915-23-7.

Student co-supervisor and frontal lessons

A.A. 2016/2017. Asia Bonciani, “Studio della morfologia pollinica di piante del Dhofar (Sultanato dell’Oman)”. Laurea triennale in Scienze Biologiche, Università degli Studi di Firenze.

A.A. 2016/2017. Fabrizio Ferritto, “Studiare il recente per interpretare il passato: presenza dell’uomo nell’antichità e suo impatto sul territorio di Pistoia con particolare riferimento agli ultimi decenni”. Laurea triennale in Storia e tutela dei beni archeologici, artistici, archivistici e librari, Università degli Studi di Firenze.

A.A. 2016/2017. Laura Tagliapietra, “Morfologia pollinica delle piante del Dhofar (Sultanato dell’Oman): gli ambienti umidi e le sabbie del litorale”. Laurea triennale in Scienze Naturali, Università degli Studi di Firenze.

A.A. 2016/2017. Michele Pampaloni, “La pioggia pollinica sulla città di Firenze”. Laurea in Scienze Naturali, Università degli Studi di Firenze.

A.A. 2017/2018. Davide Attolini, “I fitoliti nelle Poaceae: indagini nel genere *Festuca* L.”. Laurea magistrale in Scienze della Natura e dell’Uomo, Università degli Studi di Firenze.

A.A. 2017/2018. Irene Viviani, “Analisi morfometrica e micromorfologica del polline di piante del Dhofar (Sultanato dell’Oman)”. Laurea triennale in Scienze Naturali, Università degli Studi di Firenze.

A.A. 2018/2019. Pamela Rocio Marchi Brusquetti, “Morfologia pollinica di specie native di arbusti e arbusti nani sulla collina Cerro Koi, Areguà (Dipartimento Centrale – Paraguay)”. Laurea magistrale in Scienze Ambientali, Università degli Studi di Pisa.

A.A. 2018/2019. Carlotta Bambi (in corso). Laurea triennale in Scienze Naturali, Università degli Studi di Firenze.

A.A. 2018/2019. Alessia Padula (in corso). Laurea magistrale in Scienze della natura e dell’uomo, Università degli Studi di Firenze.

17/5/2017. Lezione nel corso di Botanica con Laboratorio. Laurea triennale in Scienze Biologiche, Università degli Studi di Firenze.

7/4/2018. Lezione nel Master di II livello in Progettazione paesaggistica. Dipartimento di Architettura, Università degli Studi di Firenze.

18/4/2018. Lezione nel corso di Botanica con Laboratorio. Laurea triennale in Scienze Biologiche, Università degli Studi di Firenze.

14/5/2018. Lezione nel corso di Botanica con Laboratorio. Laurea triennale in Scienze Biologiche, Università degli Studi di Firenze.

26/3/2019. Lezione nel di Botanica Forense. Laurea magistrale in Biologia molecolare ed applicata, Università degli Studi di Firenze.

16/5/2019. Lezione nel corso di Botanica con Laboratorio. Laurea triennale in Scienze Biologiche, Università degli Studi di Firenze.

Seminars

26/1/17. Il microbiota delle piante e il futuro dell'agricoltura. Dipartimento di Biologia, Università degli Studi di Firenze.

13/05/2017. La necropoli tardoantica degli Uffizi, testimonianza di un evento drammatico: indagini multidisciplinari alla ricerca della causa di morte. Soprintendenza Archeologica della Toscana, Firenze.

2017. Ecology seminars. Prof. J. Lepš and others. Quantitative Ecology module. Faculty of Science, University of South Bohemia.

22/02/2018. Governare la biodiversità: dalla cartografia al monitoraggio di habitat, animali e piante da conservare nel territorio toscano. Regione Toscana, Firenze.

12/07/2019. When India meets Italy: case studies to discuss the history of phanerozoic flora, vegetation and climate. Prof. A.K. Srivastava. Dipartimento di Biologia, Università degli Studi di Firenze.

Awards

11/7/2019. Best poster communication awards for young researchers at the Medpalyno 2019 Symposium (Bordeaux) in Aeropalynology – Pollen morphology, biology & biochemistry – Melissopalynology.